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AMUDHAKALASAM SIDDHA LITERARY RESEARCH CENTRE

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Editorial

WORLD HEALTH ORGANISATION (W. H. O.) AND TRADITIONAL MEDICINE

It is very gratifying that the World Health Organisation [W.H.O.] has now accepted traditional system of medicine as useful method for giving efficient medical and health care to the people, especially to those living in the rural areas. We must congratulate this world body for taking such a bold and revolutionary decision in the interest of improving the standard of health care to rural masses in the developing countries. It is a known fact that in the implementation of any planned health care delivery system, selection of adequately trained medical men is the most important task. For achieving this, the training pattern of medical graduates will have to be drastically changed. This will be very slow and time consuming process and will take decades before we can think of supplying adequate number of such doctors to serve the rural people.

Pending this, there is an urgent need for utilising the services of all the existing trained personnel of traditional medicine like physicians trained in Ayurveda, Unani, Siddha or Homoeopathy, for providing efficient health care to the rural masses if necessary after giving some additional trainiag. This is perhaps the only way by which the principles enunciated by W. H. O. can be quickly implemented. The world's most populous nation–China has already shown the way in improving the health care system to the rural masses by fully utilising the services of traditional physicians and bare foot doctors. They have evolved a national medical system in which best of both modern and traditional system have been fully utilised for the betterment of their people. By this process they have eradicated almost all the preventable diseases and developed efficient and ideal health care system both to the rural and urban masses. It is high time that all the developing nations should realise this and make the full use of the experiences gained in China. They should develop their own pattern of health delivery system quickly and to the entire satisfaction of both rural and urban people. For evolving such a pattern of Health Care, the W.H.O. has given its green signal and it is for the nations of third World to make full use of their wise advice.

—*K. N. Udupa*
Chief Editor

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PHARMACOLOGICAL SCREENING OF ALCOHOLIC EXTRACT OF "SARCOSTEMA BREVISTIGMA" *(SOMAVALLI)*

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[Received for Publication on October 6, 1975]

Introduction

Sarcostema brevistigma, commonly named *"Somavalli"* is a leafless trailing or twining jointed shrub, usually found on acrid rocks in Konkan, upto an altitude of 4500 ft. It is often used by farmers to extirpate white ants from sugar cane fields (Chopra, 1948). According to Kirtikar and Basu the plant is bitter, cooling, cures *tridosh*, biliousness, thirst and the dried stem has emetic properties. Though called *"Somavalli"*, this plant does not seem to be the *soma* plant of the vedic period (Nadkarni, 1954).

The stalk of this plant contains malic-acid, sugar, traces of tannins, phytosterol and terpenoids. Alcoholic extract of this plant was shown to contain alpha amyrin, bita amyrin, bita sitosterol by Beri and Sharma (1963). Gupta *et al* (1969) reported that certain triterpenoids, alpha amyrin, beta amyrin obtained from the indigenous plants possess potent anti-inflammatory, anti-pyretic and analgesic activity.

Materials and Methods

Bark of *Sarcostema brevistigma* was collected from Satara (Maharashtra State) and was identified by Botanical Survey of India, Poona. The air dried powder stem bark was extracted with 50% alcohol. The solvent of the extract was removed and the residue weighed. Aqueous suspension of the extract was prepared and was subjected to phytochemical studies. This aqueous extract was used for pharmacological screening, LD 50 was determined by i. p. route in albino mice by Miller and Tainter Method (1944).

The general behavioural changes in albino rats were studied (Sandberg Finn).

EFFECT OF EXTRACT ON DOG BLOOD PRESSURE, RESPIRATION AND INTESTINAL MOVEMENTS

10 healthy mongrel dogs of either sex, weighing between 12-14 kg were used. They were anaesthetised with pentobarbitone sodium 35 mg/kg. Femoral arterial blood pressure, respiration and intestinal movements were set up by the usual method. Dose response of the extract with doses ranging from 250 μg/kg to 8 mg/kg was obtained. Using atropine (2 mg/kg) in three divided doses of intervals of 5 minutes and mepyramine-maleate

---

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Part of the work presented here was submitted as the dissertation for M. Sc- (Pharmacology) of the University of Poona by Shri. A. L. Moholkar.

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(10 mg/kg), possible mechanism of the effect of the extract was studied.

SMOOTH MUSCLE

(a) *Guinea-pig ileum*

The preparation was set up by the usual method (Perry, 1968). The extract in doses of 100 μg/ml to 1600 μg/ml bath was added to the organ bath and graded responses were taken. After taking graded responses of the extract, a dose of 400 μg/ml bath was selected and was added to the organ bath and repeated responses of the same dose were taken. Atropine (0.6 μg/ml) and mepyramine-maleate (2 μg/ml) were tried to see the possible blockade of contraction produced by the extract. The effect of the extract in the dose of 100 μg/ml to 800 μg/ml of bath concentration was studied on isolated rat uterus and on the frog rectus abdominis muscle (Perry, 1968).

In the total dose of 400 μg the effect of the extract was observed on isolated mammalian heart by Langendorff's method (Burn, 1952).

Anti-inflammatory activity of the extract in the various doses was tested on three models in albino rats-Rat paw oedema (Selye, 1949), Cotton pellet method (Meier *et al.* 1950) and Granuloma pouch (Selye, 1953). In Rat paw oedema, the paw volumes of right and left are determined immediately, 15 minutes, 2 hours, 4 hours and 24 hours after using microburette described by Buttle and others (1947).

The antipyretic activity of the extract was tested using the technique of brewer's yeast induced pyrexia (Gujral *et al,* 1955). The analgesic activity was studied using radient heat method (Gujral and Khanna, 1956), tail pinch method (Bianchi, 1954), chemical pain method (Witkin, 1961).

Anticonvulsant activity was tested in chemically induced convulsions (Toman, 1964) and electrically induced convulsions (Handley, 1948).

Hypnotic activity (Kuhn and Vanmaanan, 1961) and local anaesthetic activity (Chance, 1944) was also studied.

The results of Rat paw oedema, cotton pellet granuloma and yeast induced pyrexia were analysed using unpaired 't' test, while the results of analgesic activity by radient heat method was analysed using paired 't' test.

Results

Acute LD 50 by i. p. route was found to be 3. 3 gms/kg body weight as calculated from the graph.

DOG BLOOD PRESSURE, RESPIRATION AND INTESTINAL MOVEMENTS

The extract in doses ranging from 250 μg/kg to 8 mg/kg showed dose dependent fall in blood pressure which an administration of atropine (2 mg/kg) showed complete blockade of the same responses (Fig. 1).

Mepyramine–maleate could not block the hypotensive effect of the extract. On isolated mammalian heart the extract in the total dose of 400 μg/ml in Guinea–pig showed negative inotropic and chronotropic effects. This shows cholinergic mechanism in the hypotensive effect of the extract.

Slight respiratory stimulation was observed in the dog. The extract does not show any significant activity on intestinal motility of the dog.

ACTION ON SMOOTH MUSCLE

From 400 μg/ml to 1600 μg/ml, the extract produced graded spasmogenic effect on guinea-pig ileum. The spasmogenic effect of the extract could be significantly blocked by atropine (0.6 μg/ml) (Table I). The spasmogenic effect of the extract is not blocked by mepyramine–maleate (2 μg/ml). This suggest

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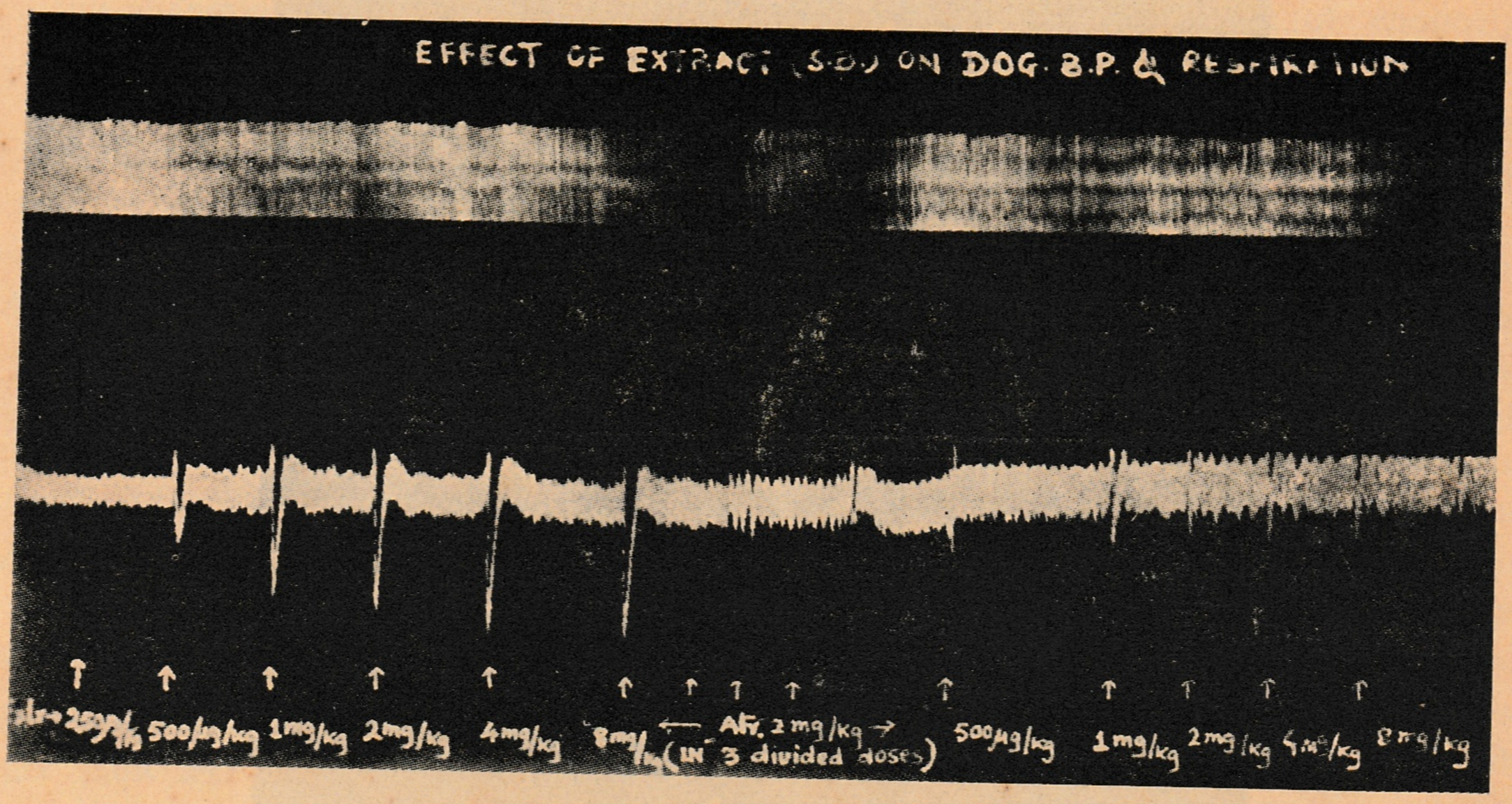

Fig. 1

Effect of extract (S.B). on dog B. P. & respiration, showing dose dependent fall in Blood-pressure which was blocked after Atropine (2nd/kg).

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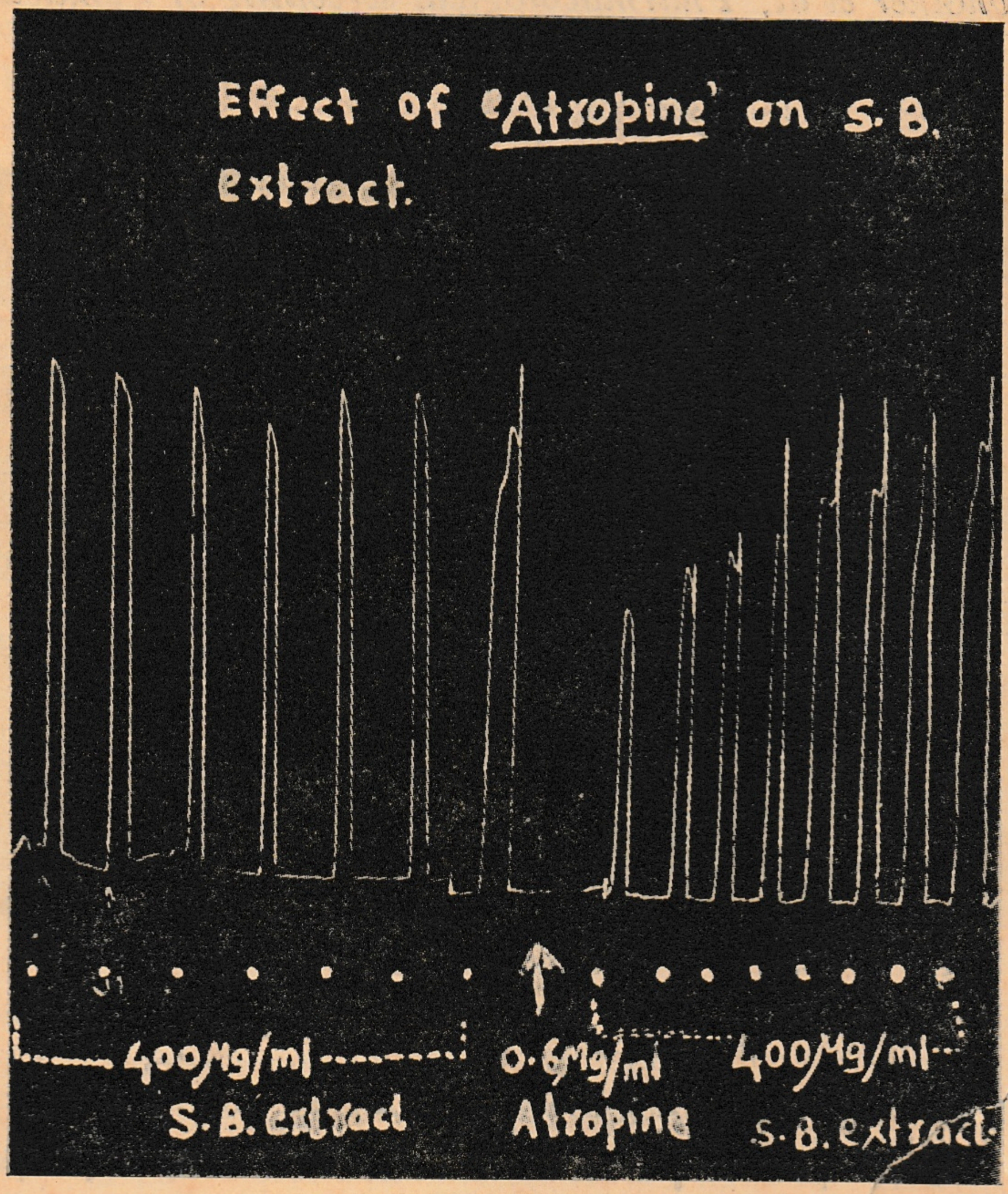

Fig. 2

Showing spasmogenic effect of the extract (S.B) (400 µg/ml) on Guineapig ileum which was blocked by Atropine (0.6 µg/ml) and showed complete recovery after repeated washing

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the involvement of cholinergic mechanism in the spasmogenic effect of the extract. By repeated washing of the tissue for 30 minutes, the recovery of the spasmogenic response of the extract is possible, after atropine blockade (Fig. 2).

The extract in the doses studied, did not produce any effect on isolated rat uterus and frog rectus abdominis muscle.

ANTI-INFLAMMATORY ACTIVITY

From the results of rat paw volume it appears that the extract suppresses the immediate phase of inflammatory process, when tested immediately 15 minutes after (Table 2). The action is of short duration, because as the time passes, the protective action of the drug disappears. There is no significant reduction is rat paw oedema after 2, 4 and 24 hours.

In cotton pellet method the drug has produced dose dependent inhibition in the formation of granulation tissue (Table 3).

In granuloma pouch method, the drug treated rats show less inflammation in the sac, no fluid in the sac and no recrosis of the underlying tissues, as compared with the control.

Analgesic activity in mice as tested by radient heat method is shown in table 4. In chemical method 70 % of mice has shown protection against acetic acid induced writhing whereas in the control group, all the mice had shown writhing.

Antipyretic activity in rats after the administration of the extract was significantly reduced (Table 5). The extract in the doses studied neither protects the rats against electrically and chemically induced convulsions, nor affect the hypnotic activity (Kuhn and Vanmaanan, 1961). It does not show local anaesthetic activity in rabbits (Chance, 1944).

Discussion

The extract produced hypotensive effect in dogs which could be blocked by atropine (2 mg/kg). It had dose dependent, spasmogenic effect on Guinea-pig ileum which could be significantly blocked by atropine and not by mepyramine-maleate. The extract produced depressed effect on isolated mammalian heart. This suggests the involment of cholinergic mechanism. The alcoholic extract of this plant has been reported to have anti-inflammatory, antipyretic and analgesic activity (Gupta *et al,* 1969), our observations on the effect of extract on formalin induced rat paw oedema, cotton pellet induced granuloma and granuloma pouch showed that the drug in the doses studied offered significant protection in the early phases of formalin induced oedema, whereas it offered less protection in the later phases of acute inflammation; significant protection in the cotton pellet and granuloma pouch method is offered showing its usefulness in inflammatory process. Histamine is known to be the chief mediator released during the early phase of acute inflammation, whereas Kinin and prostaglandin are released in the later phase (Rosa *et al,* 1971). Though the exact mechanism of anti-inflammatory action of the drug is not yet explored, from our observations it appears that the drug shows maximum anti-inflammatory activity during the first phase of acute inflammation, hence it may be interfearing with the effect of early mediaters of inflammatory reaction. In the formalin induced oedema, the drug was administered continuously for 6 days before the inflammatory challenge. In the latter stages of acute inflammatory process, the drug has failed to show any effect. This might have been due to considerable fall in the concentration of the extract much below the effective anti-inflammatory level either due to its

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TABLE 1

Effect of alcoholic extract of Sarcostema Brevistigma on Guinea-pig ileum.

Test object	Bath cons. of extract/ml.	Average response of the extract in m. m. ± S E. Before Atropine	After Atropine	Significance by 't' test
Guinea Pig ileum (6)	400 µg.	54±4.8	13±2.4	P<0.001

( ) Number in parenthesis denotes the number of preparations studied.

TABLE 2

Acute inflammation :—Rat Paw Oedema Method Rats in each group–8

Time	Dose	Mean volume of foot in ml. Left.	Right	Mean diff. in ml±SE	%oedema	Significance
15 minutes	Control	0.25±0.8	0.50±0.01	0.25±0.12	—	—
	120 mg/kg	0.34±0.01	0.51±0.01	0.17±0.08	68	—
	240 mg/kg	0.34±0 01	0.44±0.04	0.10±0.05	40	P<0.01
	480 mg/kg	0.45±0.02	0.54±0.04	0.09±0.04	36	P<0.01

TABLE 3

Cotton Pellet Method

Treatment	No. of rats	Mean weight of cotton pellets in mg per rat ± S. E.	% control	Significance
Control	5	324±54	—	
Drug 120 mg/kg	5	300±14	92 %	N. S.
Drug 240 mg/kg	5	228±16	70 %	P<0.05
Drug 480 mg/kg	5	163±18	50 %	P<0.01

TABLE 4

Analgesic activity in mice as tested by radiant heat method

Extract dose	Animal	No.	Pain threshold in sec. Mean±S. E. Before	After	Level of significance
400 mg/kg	Mice	10	8.8±1.01	15.7±2.42	P<0.05
800 mg/ka	Mice	20	8.0±0.51	23 ±1.8	P<0.001

TABLE 5

Antipyretic Activity

Effect of alcoholic extract of Sarcostema Brevistigma on yeast induced pyrexia in female rats.

Drug	Dose	Temp. of apyretic rats±S. E.	Temp. of rats±S. E.	Temp. 4 hrs. after the extract±S. E.	Significance
Control (6)	——	97±0.22	101±0.41	101±0.32	——
Extract (6)	800 mg/kg	97±0.23	101±0.03	97±0.54	P<0.01

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rapid excretion or metabolism. When continuous administration of the extract was done, after the inflammatory challenge, as in the cotton pellet and granuloma pouch, the extract has protected the animals from the chronic inflammatory process. This could be due to maintainance of the effective anti-inflammatory concentration of the drug in the body thereby supressing further stages of inflammation,

The extract showed significant antipyretic activity on yeast induced pyrexia in rats and significant analgesic activity by radiant heart method in mice.

Summary

50% alcoholic extract of *Sarcostema brevistigma* possesses hypotensive, spasmogenic, anti-inflammatory, antipyretic, analgesic activities. Acute toxicity i. p. in mice yielded approximate LD 50 value 3-3.2 gm/kg. The extract did not have any effect on isolated rat uterus and rectus abdominis muscle. Also no behavioural effects were detected. It did not show any anti-epileptic, anti-convulsant actions nor showed any modification in pentobarbitone induced hypnosis in rats. It lacked in local anaesthetic activity.

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हिन्दी सारांश

सारकोस्टेमा ब्रेविस्टिग्मा (सोमवल्ली) के औषधि गुण-धर्मों का परीक्षण

ए० एल० मोहोल्कर, एस० एम० मजुमदार एवं जी० वी० जोगलेकर

सारकोस्टेमा ब्रेविस्टिग्मा का सत्व ५०% मद्यार्क में तैयार किया गया जिससे १७% सत्त्व मिला। रासायनिक जाँच से इसमें ग्लायकोसाइड, स्टिरायड, टैनीन, सुगर, सैपोनीन तथा प्रोटीन प्राप्त हुआ। तीव्र विषाक्तता (Acute toxicity) चूहों में लगभग L D 50 3–3.2 gm/kg थी।

इस दवा का अर्क कुत्तों के रक्तचाप को कम कर दिया जोकि एट्रोपीन से अंशतः संयमित रहा। इस दवा से गिनीपिग के क्षुद्रान्त्र का आंकुचन हुआ जो एट्रोपीन से संयमित रहा न कि मेपीरेमिन मैलियेट (Mepyramine Maleate) से। इस दवा के अर्क से हृदय-गति भी कम होती रही। अर्क के सेवन से चूहों के शोथ जो भिन्न-भिन्न विधियों से उत्पन्न किया गया, में पर्याप्त कमी पायी गयी।

यीस्ट (Yeast) द्वारा बढ़े हुए शरीर के ताप को इस औषधि द्वारा कम किया गया। Radient Heat विधि से चूहों में जो दर्द उत्पन्न किया गया वह भी काफी कम हुआ।

अर्क का यूटेरस तथा रेक्टस-आब्डामिनिस मांसपेशियों पर कोई प्रभाव नहीं मिला। इस अर्क का चूहों पर आक्षेपहरण, निद्राजनन या सम्मोहन जैसा कोई प्रभाव नहीं मिला।

AMUDHAKALASAM SIDDHA LITERARY RESEARCH CENTRE

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EXPERIMENTAL STUDY ON ESTROGENIC ACTIVITY OF CERTAIN INDIGENOUS DRUGS

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Introduction

In female reproductive physiology cyclical bleeding or menstruation is first indication for her being capable of normal reproduction. It is an example in mammalion physiology of periodic sloughing from an organ. By the interplay of two ovarian hormones estrogen and progesterone the outer functional layer of uterine lining is formed and prepared to receive and nurture the fertilized ovum. In the absence of implantation hormonal support of the enriched endometrium is withdrawn. In all primates menstruation represents sloughing of this layer (Nalbandov, 1964). It is a subject for taboo, superstition and folklore and in handing on of these to young girls is responsible for many abnormal reactions to menstruation. In active cyclical life period, most women suffer one time or the other with some menstrual disorder, oligo or excess amount being the commonest symptom.

In Ayurvedic classics number of drugs have been prescribed to be useful in different menstrual irregularities amongst them *Lasun*, *Black* Til, *Ulatkambal, Changeri, Lata Karanja, Palasha Pushpa, Ghrita Kumari* are few (Chunekar, 1969; Shastri, 1966). From modern view point their probable action may be similar to ovarian hormones mainly estrogen.

Estrogens are the steriods secreted by ovarian graffian follicles during its maturation, besides graffian follicle corpus luteum, placenta and adrenal cortex are other sources for this hormone. Chief role of this hormone is related to female reproductive system, where in, it produces growth of genital organs before puberty, brings breast development and other secondary sex characteristic changes. In infraprimates it is responsible for estrus and in primates for menstrual cycle. Besides several actions on reproduction, in vaginal epithelium of infraprimates it produces typical cornification during estrus besides being responsible for vaginal opening (Allen Doisy, 1923) and its action on the uterus of mammals results in hypermia (Astwood, 1938), increase in weight of both wet and dry uterus and increased glycogen deposition (Astwood, 1939; Pincus, 1953; Spyker. 1942), which are the parameters taken to assess estrogenic activity of above mentioned drugs in present work.

Method and Material

The drugs were produced either from Ayurvedic Garden, B. H. U. or from the local market of Varanasi. Fresh expressed juice of green drugs and fine powder of dry drugs has been used. Fresh juice was dried in vacum and weighed. The doses have been expressed in terms of dry weight of juice. Part preparations and doses used are given in Table 1.

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TABLE 1

Showing Name of Drugs their part and preparations alongwith the Doses used in present work

Name of drug	Botanical name	Part used	Preparation used	Abbreviation used in further description	Doses in mgm/ 100 gm body wieght used
Lasun	*Allium sativum Linn.*	Bulb	Juice	As	150 (1cc)
Black Til	*Sepamum indicum D. C.*	Seeds	Paste prepared with water	Si	200
Ulatakambala	*Abroma augusta Linn.*	Leaf	Juice	Aa L	250 (1cc)
		root	Powder	Aa R	200
Latā Karanja	*Caesalpiniabonduc–*	Seeds	Powder	Cb	200
Ghrit Kumāri	*Roxb Aloe barba-densis. A. Indica*	leaf	Juice	Ab	150 (1cc)
Palāsha	*Butea frondosa– Roxb–Koen*	Flower	Powder	Bf	200
Chāngeri	*Oxalis corniculata– Linn.*	Whole Plant	Juice	Oc	250 (1cc)

The entire experimental study is divided in two Parts A and B.

PART–A

Effect on vaginal opening, cornification and uterus weight—Method described by Lauson *et. al.* (1939) with modification of Dorfman and Dorfman (1954) was adopted. Immature female albino rats available locally weighing between 30–40 gms and aged between 21 to 30 days were divided in 10 groups keeping 10 animal in each. Group one was given distilled water, Group two neoclinestrol in a dose of 1 mgm/100 gm body weight and remaining eight groups received above drugs in doses mentioned against each for five consecutive days. On sixth day after noting vaginal opening smear was prepared to see vaginal cornification or estrus and the animals were sacrificed, their uterine horns were separated and weighed in torsion balance. The fluid was expressed on wet blotting paper and second weight recorded. The weight of uterus in terms of per 100 gm body weight of animal was calculated and results compared statistically.

PART–B

Assessement of Myometrial glycogen contents—Six out of eight drugs showing good estrogenic activity in Part–A were subjected to this study. Type and number of animals of each group were similar to Part–A. The animals were divided in 8 groups. The drugs were fed for six consecutive days. On 7th day after sacrificing the animals, assessment of myometrial glycogen contents was done by following stages :—

1. Both uterine horns were transferred to dry and clean pre–weighed weighing tube containing 2 ml of 30% KoH solution and second weight was taken to know weight of uterine horns.

2. Now the whole thing was transferred to centrifuge tubes and heated on water bath for

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20 minutes for dissolving the uterine horns and cooled in room temperature.

3. Added 1 ml. of saturated solution of sodium sulphate and 2 ml. of 90% alcohol.

4. Heated on water bath for 10 minutes and cooled in room temperature.

5. Centrifuged for 20 minutes and drained the supernatant.

6. Dissolved the precipitate in 2 ml. of distilled water.

7. Took 1 ml. of precipitate solution added 1 ml. of distilled water to it.

8. Added 0.1 ml. of 80% w/w Phenol solution and 5 ml. of concentrated sulphuric acid to solution of stage seven.

9. Prepared blank with 2 ml. of distilled water treated with phenol and sulphuric acid.

10. Took reading in colorimeter at 490 mm with the blank set at 1000% transmission.

11. Glycogen content in mcg/mgm of uterine tissue were calculated consulting standard glycogen curve.

Results

PART–A

All the animals receiving neoclinesterol showed vaginal opening and in nine cornification of vaginal epithelium was noted. Only one of the ten animals of distilled water group showed vaginal opening which too did not have cornification. Amongst drugs tested AaR, Aah and As showed vaginal opening of maximum animals with cornification. The details are given in Table 2.

TABLE 2

Showing Number of Animals Showing Vaginal Opening and Cornification in Different Groups

Name of drug	No. of animals showing vaginal opening	No. of animals having vaginal cornification
DW	1/10	0/10
Neoclinesterol	10/10	9/10
As	5/10	3/10
Sl	7/10	5/10
Aal	9/10	8/10
AaR	10/10	10/10
Cb	2/10	2/10
Ab	5/10	3/10
Bf	2/10	1/10
Oc	4/10	2/10

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Corresponding to the results on vaginal opening etc. the drugs showing vaginal opening in maximum animals also showed highest weight of uterus both with and without fluid. Mean ± S. D. alongwith comparison with distilled water is given in Table 3

Table 3 shows that all the drugs except Bf increased considerably weight of uterus with and without fluid in comparison to distilled water group; the 't' values of AaR and AaL were higher than neoclinesterol group thus the result of AaL and AaR were again compared

TABLE 3

Showing Mean ± S. D. weight of Uterus of all Groups and Comparison with Distillea Water

Drug	Mean ± SD of weight of uterus/100 gm body weight		Comparison of weight of uterus with distilled weter	
	With Fluid	Without Fluid	With Fluid	Without Fluid
DW	104.17 ± 29.17	88.57 ± 22.91		
Neoclinesterol	293.82 ± 49.35	253.68 ± 52.13	't' 10.46*** 'P' ∠0.001	't' 9.167*** 'P' ∠0.001
As	243.01 ± 61.64	214.46 ± 56.03	't' 6.445*** 'P' ∠0 001	't' 6.577*** 'P' ∠0.001
Sl	232.67 ± 29.48	204.18 ± 33.25	't' 9.79*** 'P' ∠0.001	't' 9.053*** 'P' ∠0.001
AaL	374.19 ± 31.35	351.81 ± 31.36	't' 19.95*** 'P' ∠0.001	't' 10.08*** 'P' ∠0.001
AaR	447.97 ± 54.12	382.73 ± 51.97	't' 17.685*** 'P' ∠0.001	't' 16.37*** 'P' ∠0.001
Cb	198.05 ± 112.40	161.48 ± 91.49	't' 2.239* 'P' ∠0.05	't' 2.444* 'P' ∠0.05
Ab	179.34 ± 71.03	158.08 ± 63.22	't' 3.098** 'P' ∠0.001	't' 3.268** 'P' ∠0.01
Bf	98.81 ± 33.24	81.31 ± 28.59	't' 1 'P' 70.05	't' ∠1 'P' 70.05
OC	197.50 ± 15.85	173.08 ± 16.49	't' 8.889*** 'P' ∠0.001	't' 9.464*** 'P' ∠0.001

* Significant at 5% level of probability.
** „ „ 1% „ „ „
*** „ „ 0.1% „ „ „

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with the results of neoclinesterol group in which both these groups showed signifIcantly higher weight than neoclinesterol.

TABLE 4

Showing Comparison af AaR and AaL with neoclinesterol

Group	Weight of Uterus/100 Body Weight			
	With fluid		without fluid	
	't'	'P'	't'	'P'
Neoclines-terol vs AaR	6.665***	∠0.001	5.543***	∠0.001
Neoclines-terol vs AaL	4.352***	∠0.001	5.100***	∠0.001

*** Significant at 0.1% level of probability.

PART–B

In assessing the myometrial glycogen contents, all the test drugs were found to have more glycogen values than distilled water. Mean of glycogen contents ± S. D. and results of statistical analysis are being given in Table 5.

TABLE 5

Drug	Mean ± S. D. of glycogen contents in mcg/mg of uterine tissue	Comparison with Distilled Water 't'	'P'
Distilled water	3.21 ± 0.6	–	–
Neoclines-terol	4.84 ± 0.31	5.91***	∠0.001
As	7.40 ± 1.173	7.788***	∠0.001
Sl	5.2 ± 1.131	3.804***	∠0.01
AaL	4.23 ± 0.72	2.67*	∠0.05
AaR	4.535 ± 1.001	2.778*	∠0.05
Ab	5.16 ± 1.052	3.939**	∠0.01
OC	5.16 ± 1.77	2.556*	∠0.05

* Significant at 5% level of probability.
** Significant at 1% level of probability.
*** Significant at 0.01% level of probability.

In comparing the results of all the test drugs with neoclinesterol except As all the drugs showed insignificant difference (P 7 0.05), however, glycogen contents of As groups were higher than neoclinesterol ('t' 5.171 & P ∠0.001). Thus it can be inferred that though all the drugs increased myometrial glycogen contents, maximum increase was seen in As group followed by neoclinesterol.

Discussion

In the present work eight drugs have been tested for their estrogenic activity in experimental animals. In Part–A wherein effect was studied in vaginal opcnings of immature rats, vaginal cornification and weight of uterus, all the three parameters showed identical results viz. the drug having maximum uterine weight produced vaginal cornification and opening of maximum number of animals. On the basis of results depicted in table two and three it can be inferred that out of eight drugs maximum estrogenic activity was seen in AaR and AaL as in AaR group all animals and AaL group 9 out of ten had vaginal opening with cornification present in 10/10 and 8/10 in AaR and AaL respectively. The weight of uterus was significantly higher than even neoclinesterol (P∠0.001). Remaining six drugs showed estrogenic activity of varying degree coming in order of sequance as Si, As, Ab, Oc, Cb, & Bf. Since activity was very less in Cb and Bf these were not taken in Part–B study.

In Part–B all the test drugs showed increased myometrial glycogen contents in comparison to distilled water. As showed even more glycogen contents than neoclinesterol (P∠0.001) Sl and Ab were next to As followed by AaL and AaR and Oc.

There seems to exist some discrepancy in the observation of different parameters of all the drugs as in Part–A AaL though had more

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uterine weight than neoclinesterol but showed vaginal opening and cornification in lesser number of animals. Similarly, As which did not show maximum estrogenic activity in Part–A had highest glycogen contents of uterus. Similarly AaR and AaL having best results in Part–A trailed much behind in Part–B. This may be possible because of biological variation of different animals as the studies were performed in two different groups. Moreover, the methods adopted are best method for qualitative study not for quantitative. Thus it can be if inferred that out of all 8 drugs *Lata Karanja* (Cb) and *Palasha Pushpa* (Bf) possess very negligible estrogenic activity, remaining six drugs have good estrogenic activity of varying degree.

Summary and Conclusion

Eight Indigenous drugs namely *Lasuna* (As), *Black Til* (Si), *Ultakambal* leaf (AaL) *Ulatkambal* root (AaR), *Latakaranja* (Cb). *Ghrita Kumari* (Ab), *Palashapushpa* (Bf), *Changeri* (Oc) have been tested for their estrogenic activity. Amongst them Cb and Bf showed very negligible effect while remaining all drugs had estrogenic activity of varying degree.

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●

हिन्दी सारांश

कतिपय औषधियों के ईस्ट्रोजेन सदृश प्रभाव का प्रयोगात्मक अध्ययन

प्रेमवती तिवारी, हे० च० मापा एवं च० चतुर्वेदी

प्रस्तुत कार्य में लसुन, काला तिल, उलट कम्बल, (पत्र व मूल), चांगेरी, लता करन्ज, पलाश पुष्प एवं घृत-कुमारी के ईस्ट्रोजेन सदृश प्रभाव का अध्ययन अप्रगल्भ मूषिकाओं के योनि-द्वार-प्रादुर्भाव, योनि की श्लेष्मल-कला परिवर्तन (ईस्ट्रस) व गर्भाशय भार आदि को आधार मान कर किया गया। संतोषजनक परिणाम प्रदर्शित करने वाली औषधियों का पुनः गर्भाशय-पेशी के ग्लाइकोजेन की मात्रा पर प्रभाव देखा गया जिसमें यह पाया गया कि उलट कम्बल मूल व पत्र में सर्वाधिक ईस्ट्रोजेन सदृश गुण हैं। पलाश पुष्प व लताकरन्ज में यह गुण नगण्य मात्र पाया गया। अन्य सभी औषधियों में विभिन्न परिणाम की ईस्ट्रोजेन सदृश क्रियाशीलता पायी गयी।

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ISOALTION OF QUERCETIN FROM *ASPHODELUS TENUFOLIUS*

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(Received for publication on June 14, 1975)

Introduction

The plant *Asphodelus tenufolius* (Chopra et al. 1952) commonly known as ·Bokat' in Hindi belongs to the natural order, Liliacene, Ayurvedic system of medicine described the plant seeds as diuretics. Medicinally it is used externally for curing ulcers and inflamed parts.

The drug plant is a weed and cultivated widely in Indo-Gangetic Plain from Bengal to Punjab.

Materials and Methods

About 3 kg. of air-dried and powdered drug was extracted with petroleum ether in a Soxhlet extractor for several days. The solvent was removed from the extract by distillation under reduced pressure when about 20 g of a brown sticky mass was obtained. The study of this mass has been communicated separately (Saxena and Singh, 1975).

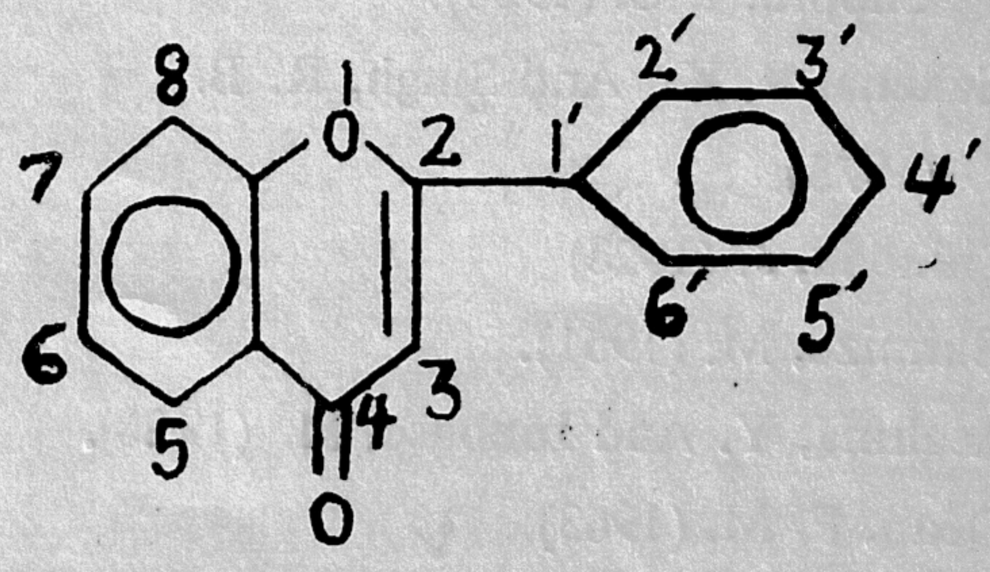
ISOLATION OF THE FLAVONOID COMPOUND QUERCETIN

The defatted drug was extracted with ethyl alcohol and the solvent removed in vacuum at 58° and the concentrated solution allowed to stand when a green yellow deposit was obtained. The deposit was repeatedly crystallised from a mixture of acetone and methanols, till a single spot was obtained by paper and thin layer chromatography. The yellow compound melted at 316°. It did not give a positive Molisch's test, but its ethanolic solution responded to the following reactions for a 3–hydroxy flavone which was further confirmed by its λmax at 256 mμ and 371 mμ.

(i) Dull green colour with ethanolic Fe Cl_3.

(ii) Various shades of red colour with Mg/HCl (Shinoda, 1928). Zn/HCl (Shimizu, 1951) and sod. amalgam and HCl (Asahina and Inubuse, 1928).

From the above reactions, it was obvious, that the compound is a flavone derivative and should have the following skeleton :



The presence of a hydroxyl group at 3–position in the molecule was shown by the fact that with ethanolic boric acid and sodium acetate it responded to a positive test (Dean, 1963), which was further confirmed by a positive reaction with zirconium oxychloride in

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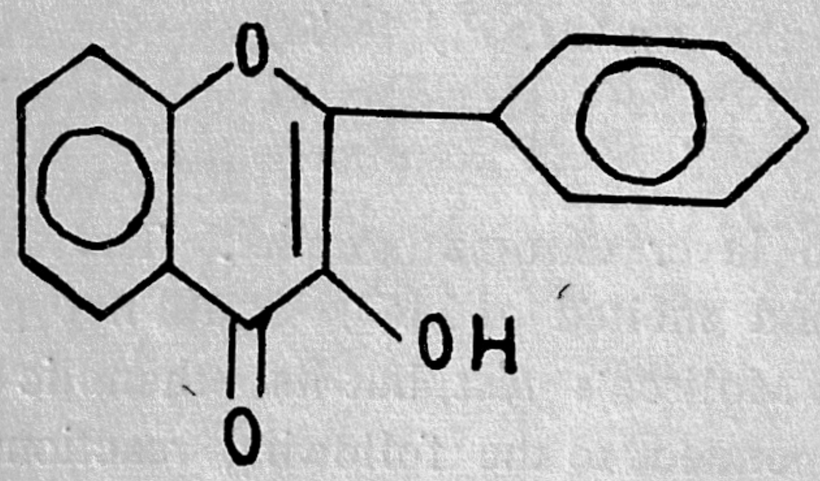
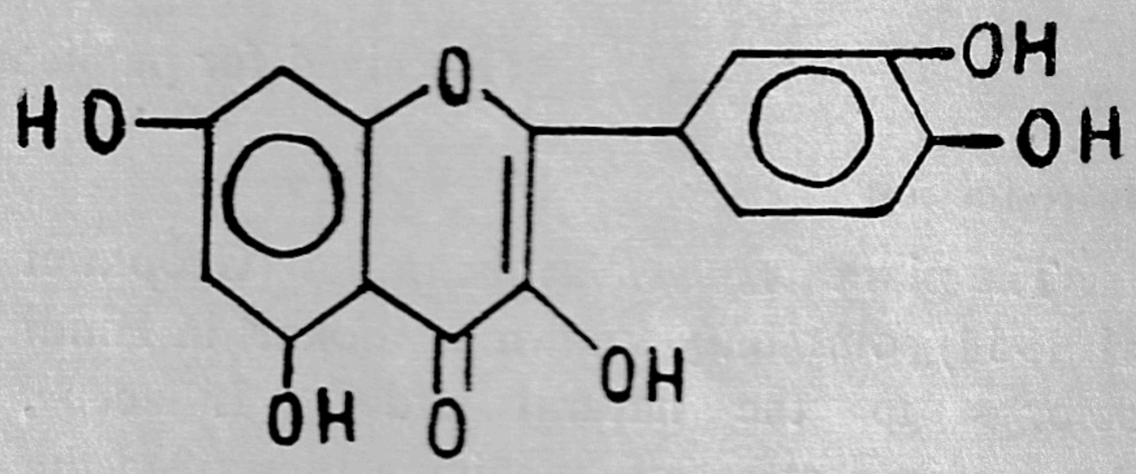
presence of citric acid (Horhammer and Hansel, 1955).

With acetic anhydride and pyridine, the compound formed a penta—acetate showing the presence of five hydroxyls out of which one–OH occupies the position–3. The compound can thus be represented as :



The compound formed a yellow fluorescent (Taubock, 1942) solution with boric and oxalic acids in acetone showing the presence of free hydroxyl at position–5. Also a positive reaction is given with vanilline-hydrochloric acid reagent (Hills and Urbach, 1958) showing the presence of one hydroxyl at position–7.

A bathochromic shift of 18 mμ in the visible region on addition of boric acid–sod. acetate reagent (Jurd, 1956) to the ethanolic solution of the compound indicated the presence of free hydroxyls in positions–3' and–4'. Hence the compound has the structure :



The structure represents the well known compound quercetin which was finally confirmed by mixed melting point and co-chromatography with an authentic sample of quercetin.

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हिन्दी सारांश

क्यूरेसिटीन का एस्फोडिलस टिन्यूफेलियस नामक वनस्पति से पृथक्करण

बी० के० सक्सेना एवम् आर० बी० सिंह

एस्फोडिलस टिन्यूफोलियस भारतीय चिकित्सा प्रणाली में बहुतायत से प्रयुक्त होने वाली औषधि है। हिन्दी में उसे बोकत कहते हैं। आयुर्वेदिक औषधि प्रणाली में इसका उपयोग मूत्रवर्धक औषधि के रुप में किया जाता है। बाह्य-रूप से इसका उपयोग घाव तथा जले हुए शरीर के अंगों को अच्छा करने में होता है। इसके पंचांग से विविध रासायनिक अभिक्रियाओं द्वारा एक महत्वपूर्ण फ्लैवीन, क्यूरेसिटीन प्राप्त किया गया है।

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PHARMACOGNOSTICAL AND PHARMACOLOGICAL STUDIES ON *TERMINALIA ARJUNA*

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Introduction

The Ayurvedic drug *"Arjuna" (Terminalia arjuna)* is a large deciduous tree, attaining a height of 60 to 80 feet with huge, often butteressed trunk and horizontally spreading branches. Its bark is smooth, pinkish grey flaking off in large flat papery pieces at certain places (Fig. 1). It is common throughout the sub–Himalayan tracts, South Bihar, Chhota Nagpur, Madhya Bharat, parts of the eastwhile Bombay and Madras states. Out side India, it is found in Burma and Ceylone.

According to Ayurveda, the bark is acrid and sweet in *'Rasa'*, cooling in *'Guna'*. It is credited with several therapeutic properties both in Ayurvedic and 'Unani' systems of medicine.

Among the ancient Indian authorities on the medical science 'Vagbhatta' was the first, who prescribed the bark of *Arjuna* in heart diseases (Astānga sangraha with Arundutta Comm., 1939). Later 'Chakradutta' the great Hindu physician, described it as a tonic astringent and used it in heart diseases. He recommended it to be given as a decoction with milk and treacle water or as Ghrit. The bark powdered with honey is also recommended to be given internally in fractures and contusions with echymosis, in bilious disorders, diarrhoea, dysentry, sprue and haemorrhages (Chakradutta, 1961). The decoction is also useful as an astringent for cleaning sores and ulcers etc. (Charaka Samhita with Chakrapāni comm., 1941). Bhāva Prakāsh, a Nighantukar has also recognised the bark as possessing some cooling, cardiotonic, wound healing and intoxicating effects (Bhāva Prakāsh, 1969).

Among the modern investigators on chemistry and pharmacology of the drug, Hooper (1891) was probably the first who reported the presence of calcium salts and tannins in the bark. Later on, Ghosal (1909) investigated the bark chemically and reported the presence of sugar, tannin—a colouring matter, glucosidic principle, carbonates of calcium and sodium with traces of chloride and alkali metals. Based on his own pharmacological studies on frogs, rabbits and men, he accepted the drug to be a valuable remedy in heart diseases, specially where combined tonic and stimulant action is necessary. In 1923 Chopra also reported pure glucoside from the bark and recognised that it produces a small but persistant rise of blood pressure in experimental animals.

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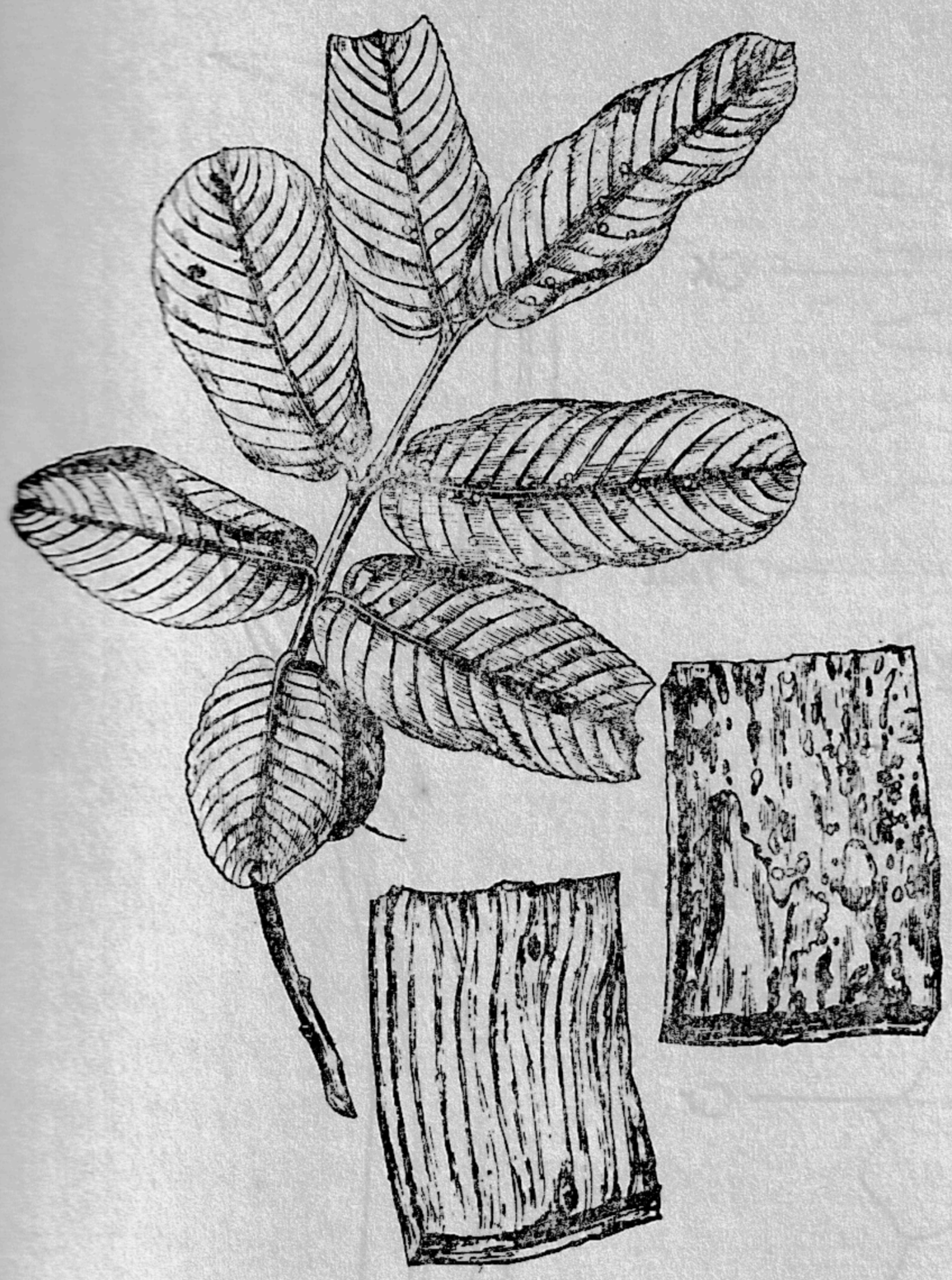

Fig. 1

Shows the bark and a twig of *Terminalia arjuna*. 'A' is the dorsal view of the bark which is smooth, pinkish grey and flaking off in papery pieces at certain places. 'B' is the redish white, ventral appearance of the bark and 'C' a twig having sub–opposite leaves, 10–15 by 4–7 cms oblong.

On clinical trial, the total alcoholic extract of the drug was found to have beneficial effect on cardiac cases. who did not respond well to digitalis therapy (Chopra and Ghos, 1929). Recently, the powdered bark has been said to relieve hypertension and also to have a diuretic and general tonic effect in cirrhotic cases and on chemical analysis, the presencc of calcium, arjunilic acid, saponin and leuco-delphinidin have been reported (Chopra, 1969)

From these pharmacological investigations, it appears that the isolated glucoside has a little hypertensive property while the total alcoholic extract and the powdered drug has a cardiotonic and hypotensive action in clinical subjects in addition to this discrepancy, the literature also lack in mentioning the specific indications of the drug in cardlac ailments. Hence, in view to re–investigate the pharma-codynamic property of the drug, specially on cardio–vascular system in general and coronary occlusions in particular, the study has been aimed. The indication of the drug, if any, on coronary occlusion was evaluated by measuring the prothrombin time,

Material and Method

I. IDENTITY OF TERMINALIA ARJUNA

Sample of the drug was procured locally from Banaras Hindu University Campus. for the pharmacological investigations. The macro-scopic and microscopic studies (Fig. 2 A, B & C) revealed the characteristics, which were in confirmity with the findings of S. Pra-sad (Prasad, 1941).

II. PRELIMINARY PHYTOCHEMICAL STUDY :

A preliminary study was undertaken to estimate extractives of the drug in different solvents, ranging from non-polar solvent to polar-solvent and also to assess qualitatively, the nature of proximate principles present in these extractives.

Freshly collected bark of *T. arjuna* was dried under shade and about 150 gms. of dry bark was powdered to a moderate coarse pow-der (No. 10 powder). This was packed in soxhlet extractor and successively extracted by hot continuous extraction (Soxhlet) method with petr oleum ether (60°–80°), benzene, chloroform and ethyl alcohol. The different

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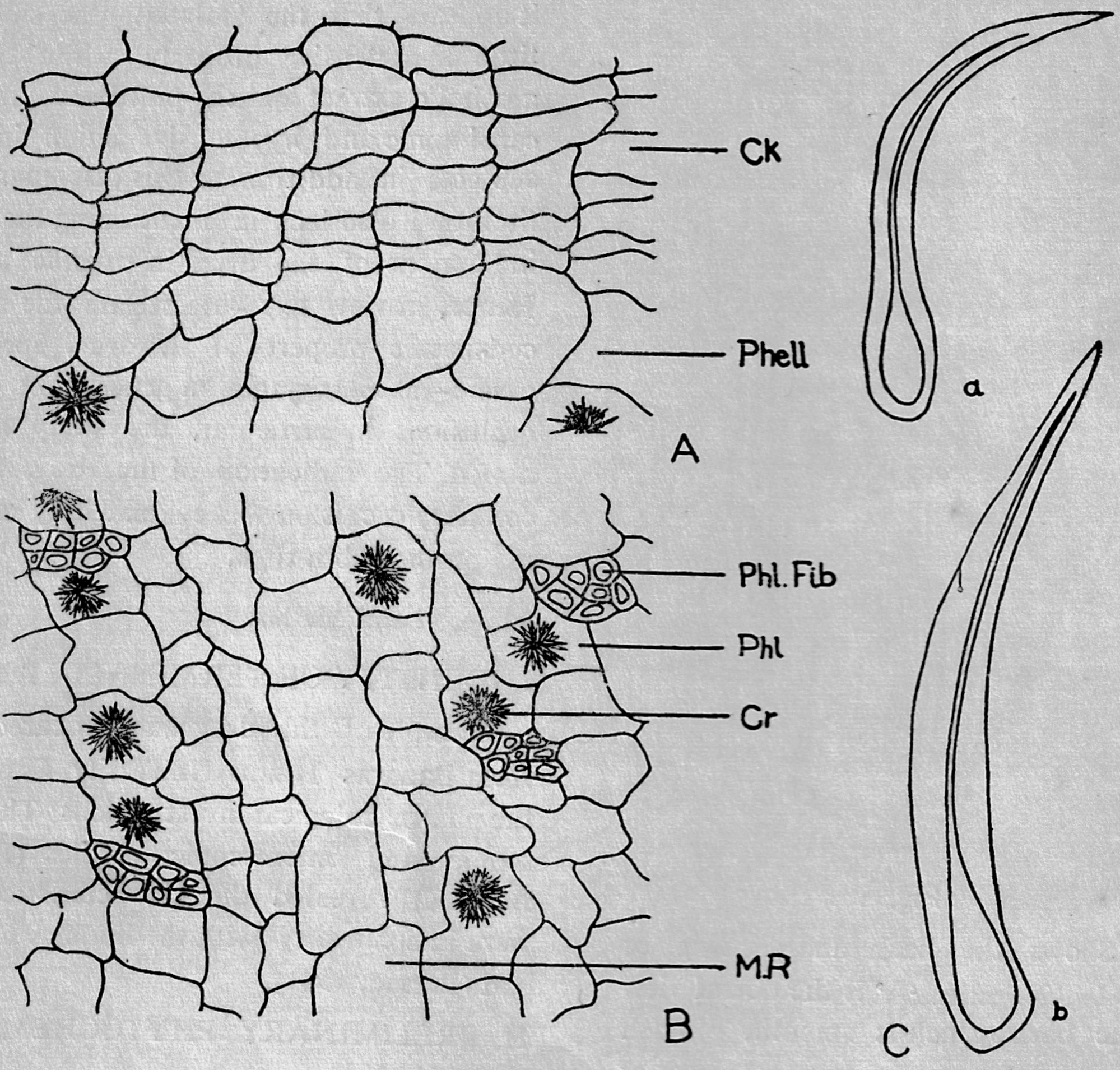

Fig. 2

A camera lucida tracing of microtome–section of stem–bark of *T. Arjuna*. 'A' shows few layers of cork cambium and secondary ploem only. 'B' a mature bark consisting of a very broad zone of uniseriats phloem rays, phloem fibres crystal fibres seive tubes and phloem parenchyma. The crystal fibres, contains rosett crystals of calcium oxalate. 'C' a pair of combretaceous type of hair having swollen base and tapering pointed apex. It varies in lenght, thickness and width of the wall 'b'.

extractives were tested for the presence of alkaloids, glycosides, free reducing sugar and sterols as per approved methods (Peach, *et al.*, 1955; Rosenthaller, 1930). The different extractives showed the presence of the following active constituents.

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TABLE 1

No.	Extractive	Chemical constituents present			
		Alkaloid	Glucoside	Reducing sugar	Sterol
1.	Petroleum Ether	—	—	—	+
2.	Benzene	—	—	—	+
3.	Chloroform	—	+	+	—
4.	Alcohol	—	+++	+++	—

III. PHARMACOLOGICAL INVESTIGATIONS

The water soluble portion of the total alcoholic extract of the drug was used for studying the pharmacological activity of the drug. A preliminary chemical analysis of this preparation showed that it contained reducing sugar, some phenolic compounds, tannins and inorganic salts of Potassium, Sodium and Calcium.

Obseration and Results

A. EFFECT OF WATER SOLUBLE PORTION OF THE TOTAL ALCOHOLIC EXTRACT OF T. ARJUNA ON CARDIO VASCULAR SYSTEM—

(i) Perfused frog heart preparation—The water soluble portion of total alcoholic extract (A. E./Aq.) of *T. arjuna* was tested on frog heart by the conventional perfusion method. The aqueous suspension of the water soluble portion of alcoholic extract was used in a concentration of 3.2 mgm/ml and was administered in doses of 0.25; 1.0 and 2.0 ml. It was observed that the preparation in all the doses increased the force of contraction of heart (Fig. 3). The result suggested that the drug possesses cardiotonic activity.

(ii) Effect on arterial blood pressure and respiration in anesthetised dog—Dogs weighing between 6–8 kg., under Nembutol anesthesia (25–30 mgm/kg. wt.) were used in this study and the drug was administered in graded doses through femoral vein as per the conventional method. The effect of the drug on respiration and arterial blood pressure (carotid) by means of Mary's tambour and mercury manometer respectively was estimated and recorded kymographically (Fig. 4.). The results are as tabulated below :—

TABLE 2

The effect of T. arjuna on B. P.and Respiration

No.	Initial rate of Respiration/mt.	Initial B. P. mm. Hg	Dose (ml.)	Respiration rate/mt.	Blood pressure mm. Hg
1.	10	100	0.5 ml (1.6 mg)	16	100/80
2.	16	100	1.0 ml (3.2 mg)	16	100/80
3.	16	100	1.5 ml (4.8 mg)	16	100/60
4.	16	100	2.0 ml (6.4 mg)	16	100/55

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The rate rhythm and force of contraction of heart seemed to remain unaffected.

The experiment, however revealed that while the drug did not show any significant effect on respiration, there was a marked variability in the sequence of the blood pressure, specially towards the lower limit. If it is presumed that the upper and the lower limits are the only resultants of simultaneous systole and diastole of the heart seems to be markedly influenced by the drug. It is suggestive of an increased intake and naturally with this result, under a constant pressure, there would have been an increased output also, as no trace of distension could be noticed in the heart throughout the period of experimentation.

B. EFFECT ON CARDIAC OUTPUT

In order to confirm the effect of the drug on the cardiac output, a modification of frog-heart perfusion experiment, using a drop counter (Inchley's) was adopted. The frog Ringer's solution was perfused in usual manner and made out through a small nick in one of the aortas fitted with cannula. To measure the pumped-out fluid, this cannula, with a suitable length of tubing, was connected to a silver drop tube. The distance betwee the drop-tube and the silver wire was so adjusted tha tdrop touches the wire while leaving the tube. Two wires, coming out of the drop-tube assembly, were connected to the input terminals of the recorder with a special arrangement for counting the drops and the output terminals for operating the marker on the kymograph paper.

When input terminals were short circuited by the drops (pumped out by the heart) the thyratron valve gets triggered to conduct the current and energize the counter and thus the number of drops passed on are recorded. As soon as the drop touches the silver wire, the output becomes available and persists until the drop leaves the silver wire; the external marker starts working and records the time-interval in between the successive drops as the time taken by the drop to pass.

Normally, the output of the frog heart as recorded by the counter was 13 drops/mt, and the time interval in between the two drops was bigger, as became evident from the tracing. The beating rate was 18/mt. and the rhythmic contractions were regular. The A. E./Aq. (3.2 mg/cc. Conc.) of *T. arjuna,* when given in the dose of 0.25 mg. along with the perfused fluid, the output increased to 26 drops/mt. It went to 43 to 57 drops/mt. without affecting the rate of contraction following the admini stration of the drug in 0.5 and 1.0 cc. respectively. The time interval started shortening in such a quick successiong that ultimately it was found difficult to avoid the duplication of the marker (Fig.5).

The study concluded that in frog the A.E./Aq. of the drug, without altering the rate and rhythm of heart, significantly increases the cardiac output.

C. EFFECT ON DOG-HEART

A dog-heart preparation according to the conventional method was made and the normal tracings of both the auricular and the ventricular contractions were recorded. The water soluble portion of the alcoholic extract of *T. arjuna* (3.2 mg/cc. Conc.) when administered intravenously in 2.5 cc. dosage, the forceful contractions of the heart got slightly accentuated. The findings became more evident with the dose of 5.0 cc. and a significant change in tracing pattern became visible on administration of 10 cc. of the drug (Fig. 6). Thus, these findings substantiated the view that the drug on intravenous administration definitely increases the force of contraction of both the

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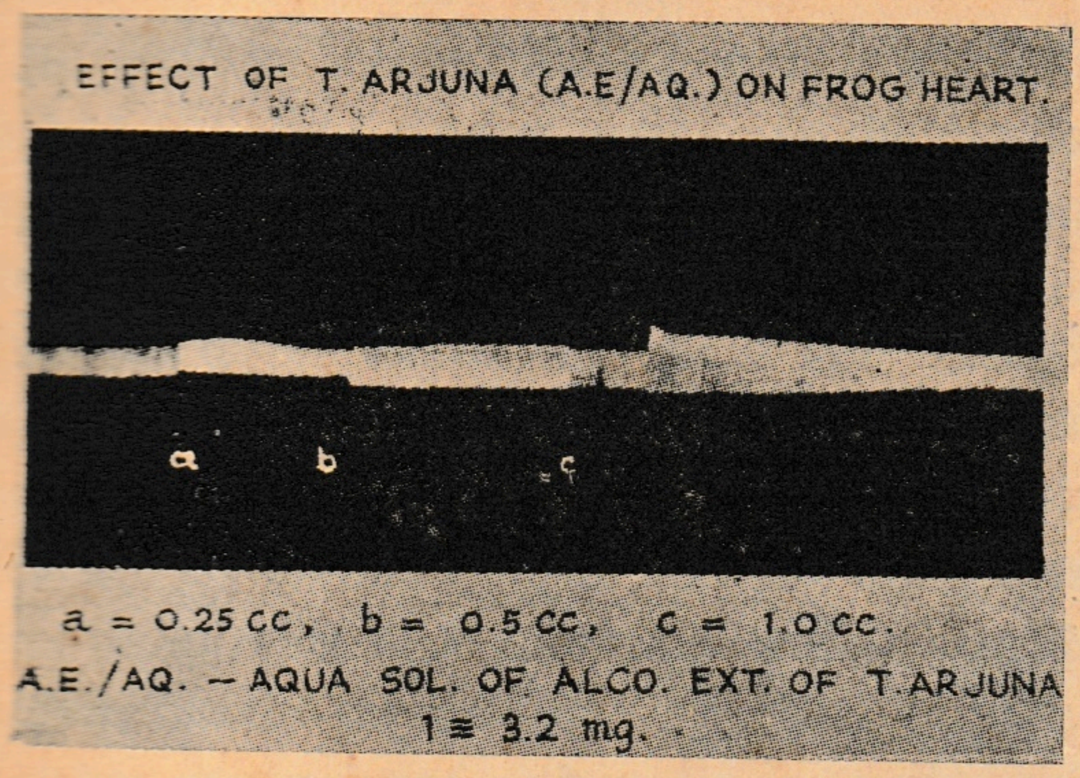

Fig. 3

The kymograph tracing showing the cardiotonic effect of *Terminalia arjuna* (AE/Aq) on frog heart, in various dosage, by perfusion method.

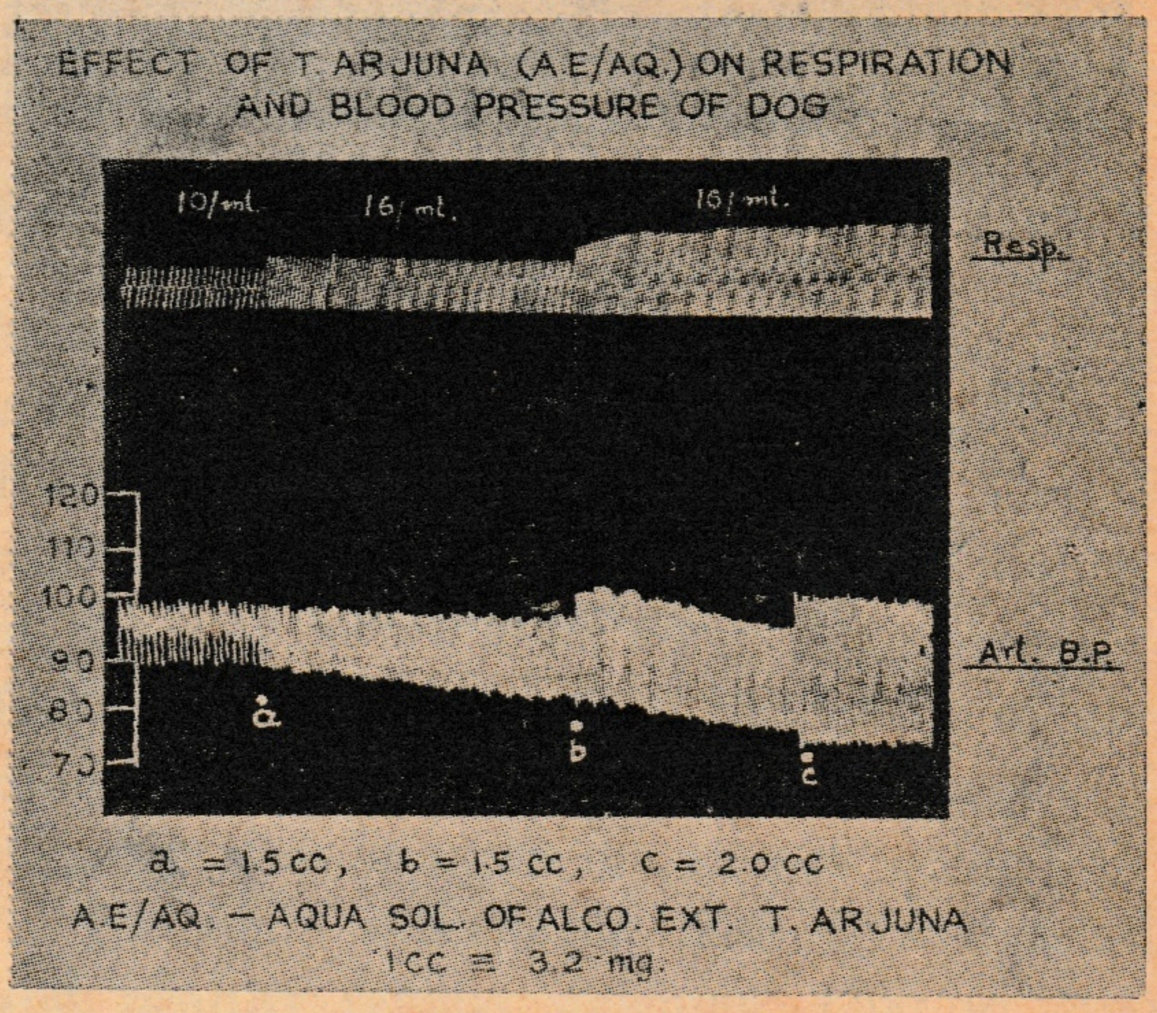

Fig. 4

Kymograph tracing ellustrates the effect of *T. arjuna* (AE/Aq) in various dosage, on respiration and blood pressure of the dog.

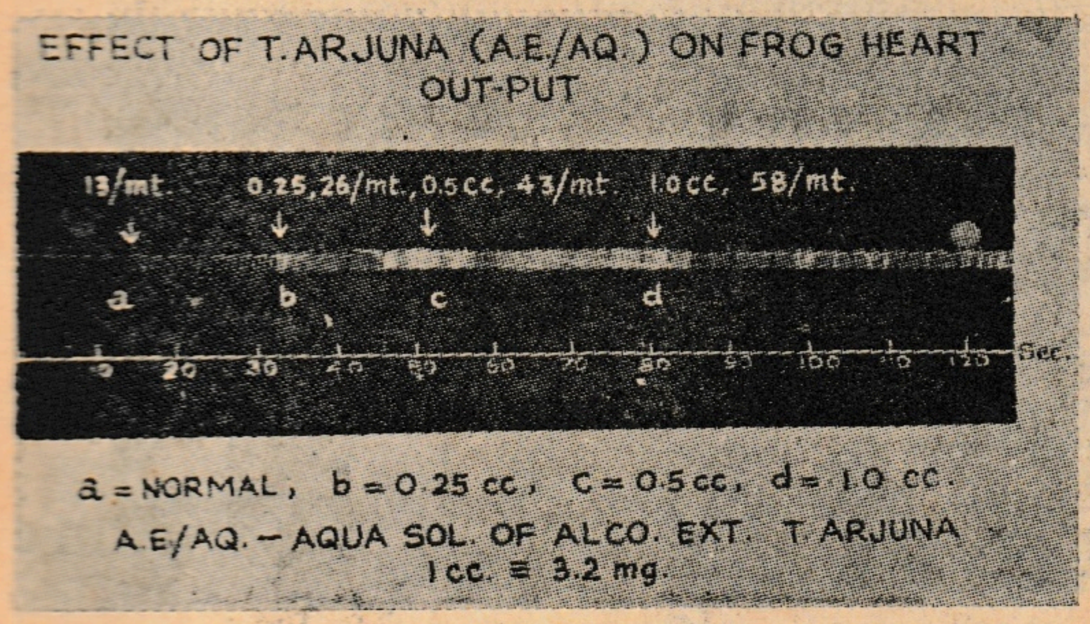

Fig. 5

The tracing shows an increased output response of the frog heart by administering the drug *T. arjuna* (AE/Aq) in different doses.

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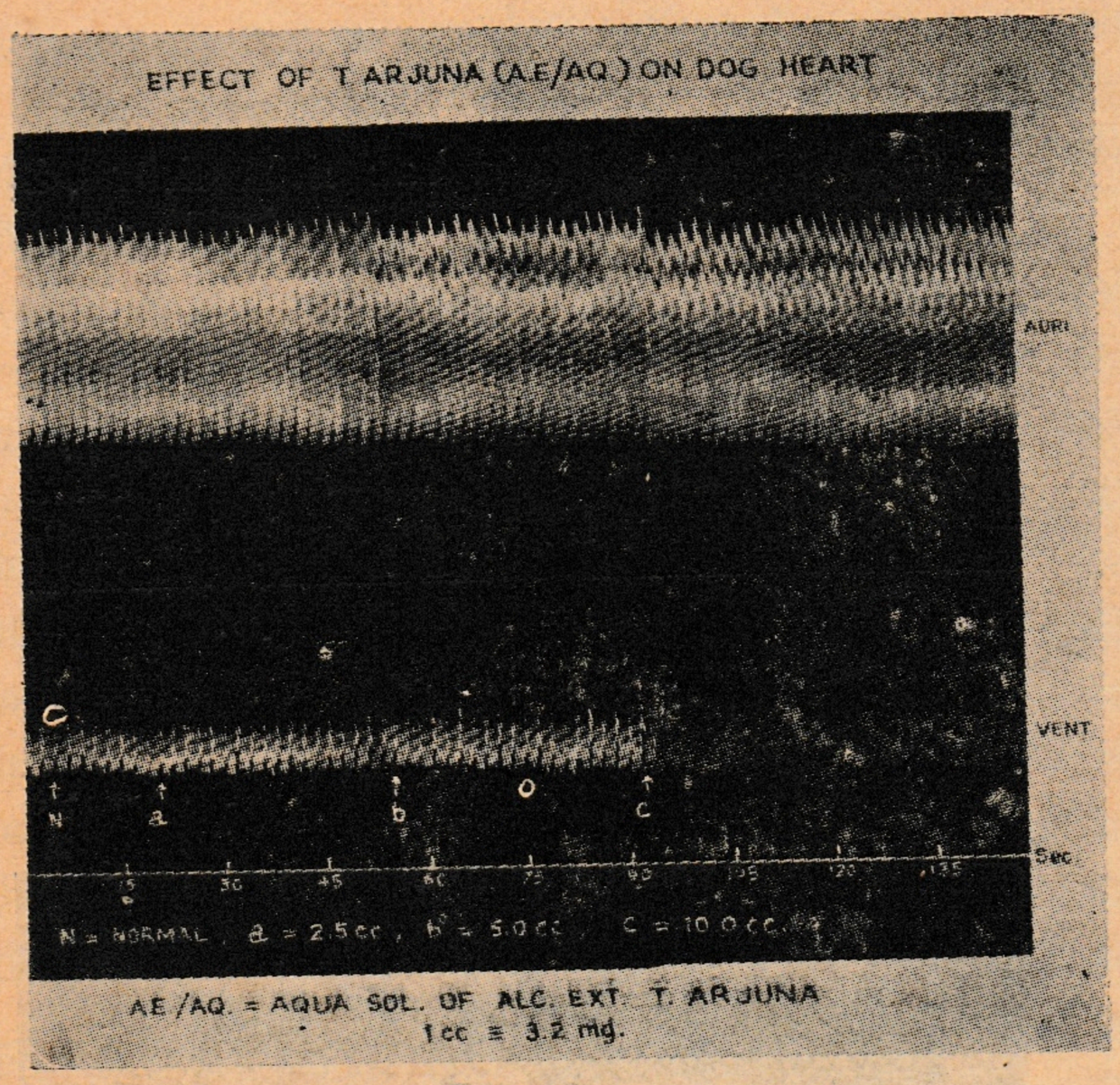

Fig. 6

The tracings denote an increased auricular and ventricular contractions of the dog heart under the effect of *T. arjuna* (AE/Aq.)

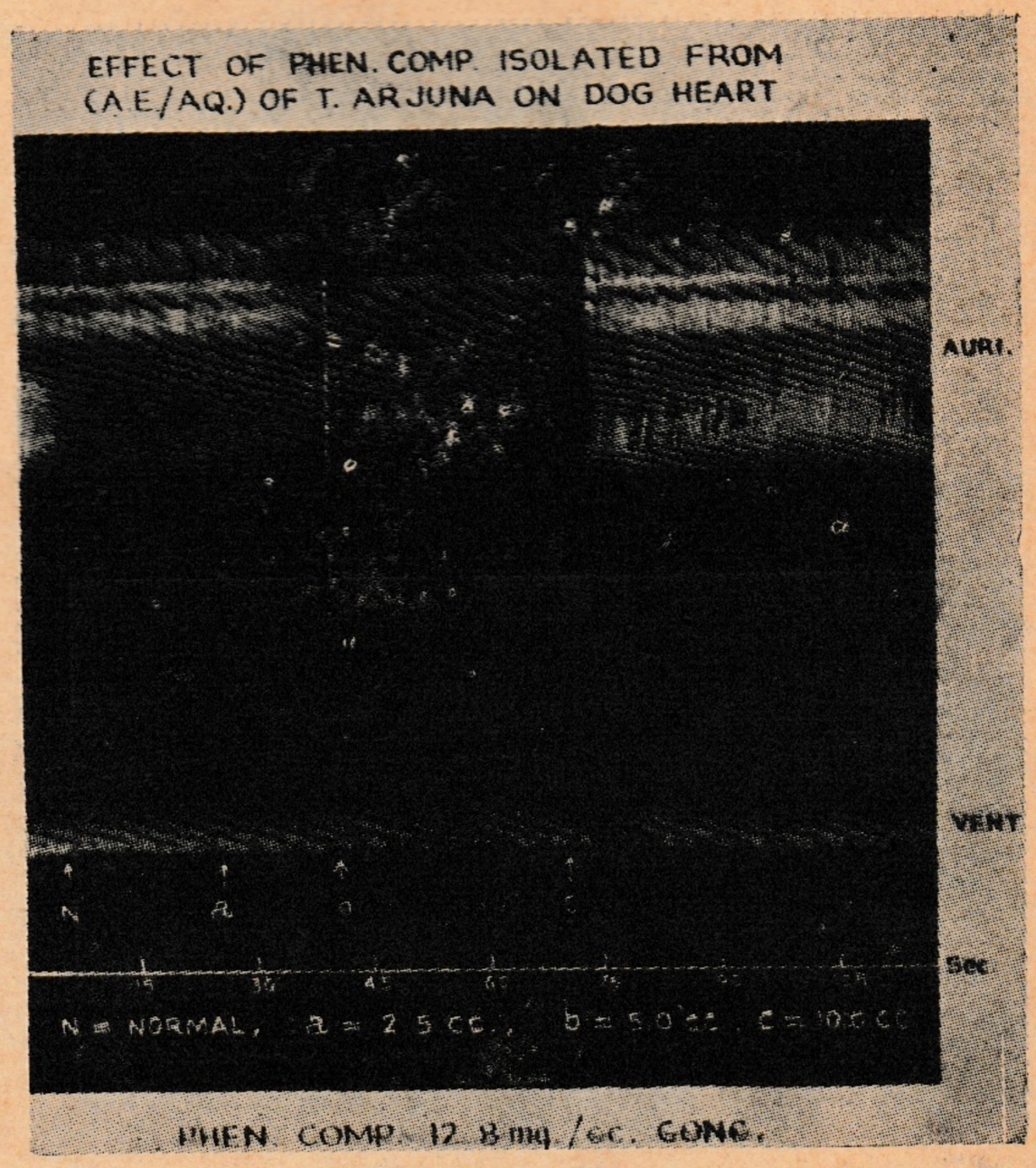

Fig. 7

The tracing shows on innert response of the dog heart against the phenol compound isolated from the whole Alcoholic ext. of *T. Arjuna*.

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auricles and ventricles, and thereby helps in advancing the cardiac output.

In order to ascertain the principle which might be responsible for enhancing the cardiac output and thereby, producing the cardiotonic effect, the A. E./Aq. portion of the drug was subjected to chemical analysis. The solution on testing for alkaloid, Glucosides and Sterol, showed negative reactions. However, the presence of reducing sugar and phenolic compounds, tannin and inorganic salts could be detected in the solution.

The presence of phenolic compound in the A. E./Aq. portion of the drug, raised a suspicion regarding its irritant action which might have caused the forceful contractions of the heart. Hence, to remove the doubt, the phenolic compound was isolated from the solution and this was tested on dog-heart preparation. The isolated phenolic compound, however, failed to increase the cardiac output or to induce any effect on heart (Fig. 7). This finding limited the scope of speculations only to the inorganic salts, i. e. calcium (Ca), potassium (K) and sodium (Na), which might be responsible for forceful contractions of the heart. About 2.0 cc. A. E./Aq. portion of the drug, when mixed with an equal amount of 4% ammonium oxalate solution, failed to give even a trace of precipitation and excluded the possibility regarding the presence of calcium. Another portion, when subjected for estimating the presence of ions, particularly the 'K' and 'Na' by Flame Photometer, an adequate concentration of both the potassium (160.0 m. equi) and sodium (120·0 m. equi) could be detected. These findings satisfied the entire possibilites lying behind the forceful contractions of the heart and indicated the presence of sodium and potassium salts.

D. EFFECT ON PROTHROMBIN TIME

Although the available evidences have failed to support the view that the coronary thrombosis is caused by an increased coagulability of blood, yet the patients with coronary heart disease have been reported to have an increased coagulability of the blood as determined by platelet stickiness, (Murphy *et al.*, 1962), thromboplastin generation, estimation of fibrinogen, prothrombin time and whole blood-coagulation time, (Katz *et al.*, 1963). A significant reduction in lipoprotein lipase activity was found in men with ischemic heart disease in comparison with normal subjects (Slack *et al.*, 1964). Further, there was a significant correlation between the activity of lipoprotein lipase and platelet stickiness in patients as well as healthy persons

Opinions differ regardlng the significance of the various blood clotting factors in causing hypercoagulability of blood in patients with coronary and other occlusive arterial diseases. However, the value of prothrombin time is considered to be of importance in pathology of subjects proved to be coronary thrombosis. Experimental evaluation of the effect of the drug *T. arjuna* on prothrombin time was also done to assess its merit in coronary occlusion.

The study was started with sixteen adult and healthy rabbits weighing 1200 to 1500 gms. They were divided into two groups of treated and control, each consisting of equal number of animals. They were freely accommodated and supplied with usual laboratory diet. Before the ingestion of the drug the two groups of animals were examined for normal value of prothrombin time by a modified Quick's method (Quick's, *et al.*, 1935). By means of a rubber catheter, one group of animals (experimental) received a dose of o. 5 gms. *T. arjuna* extract powder, suspended in water on alternate days for 30 days. The second group of animals (control) was given only same quantity of water. In continuation

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of the drug feeding or water ingestion, a periodical check up of "Prothrombin Time" in both the groups of animals on the 5th, 10th, 15th, 20th; 25th and 30th day intervals, was made. The findings were as follows :—

TABLE 3

Prothrom. Time in sec. in Drug Treated Animals

	5th day	10th day	15th day	20th day	25th day	30th day
	10.5	8.0	7.5	7.0	7.0	7.0
	9.5	8·5	8.0	7.5	7·5	8 5
	8.5	9.5	8.5	8.5	7 5	8.0
	10.0	9.0	8.0	8.0	7.0	8.0
	10.5	8.0	8 0	7.0	7.0	7 5
	10.0	8.0	7.5	7.5	7.5	8.0
	9.5	9.0	8.5	8.0	8.0	8.5
	9.0	8.5	9.0	8.5	7 5	8 0
Mean	9.7	8.56	8.12	7.75	8.43	7.94
S. D.	± 0.703	± 0.563	± 0.519	± 0.597	± .355	± 0.496

Normally, in rabbits used for this study, the prothrombin time varied from 9 to 10 seconds and the administration of the drug extract powder for a period of one month, evidently denoted a tendency of little reduction rather than the prolongation in prothrombin time.

Thus it can be concluded that the powder of whole alcoholic extract of *Terminalia arjunu,* on oral administration in rabbits did not reflect any noticeable change in prothrombin time. The water soluble portion of the drug, irrespective of the changes in respiratory pattern, however, definitely toned up the contraction power of the heart and accentuated both the auricular and ventricular contractions. Hence, for the sake of its efficacious and definite roles, the water soluble portion and the whole alcoholic extract of the drug still deserve well planned clinical trials in various conditions of the cardiac ailments.

Summary

1. *Terminalia arjuna* is a large deciduous tree, found in greater part of the India. In both the Ayurvedic and Unani systems of medicine though the bark has several indications but its fresh decoction in milk is most preferably prescribed in cardiac ailments.

2. A fresh piece of bark macroscopically represented grey and smooth appearance of outer surface with exfoliating paper cork at certain places. On sectioning, the bark showed an epidermis with combretaceous type of hairy covering followed by a few layers of collenchyma, broad zone of parenchyma and deeply aimed cork cambium in the cortex.

3. Phytochemically, the petroleum ether and Benzene extracts gave an indication for the presence of sterol, while in chloroform and alcoholic extracts the presence of both the reducing sugar and glucosides were in traces and abundant respectively.

4. (a) On bio–assay, the frog heart preparation showed an increased contraction power of the heart with water soluble portion of the whole alcoholic extract.

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(b) There was no significant response on the pattern of respiration while the cardiac intake and output was found to be markedly influenced.

(c) The water soluble portion, on intravenous administration in dogs, accentuated both the auricular and ventricular contractions probably due to adequate concentration of potassium ions.

(d) The alcoholic extract powder of the drug, on oral administration in rabbits did not exhibit the noticeable changes on prothrombin time.

ACKNOWLEDGEMENT

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हिन्दी सारांश

'अर्जुन' का द्रव्य-संरचनीय और द्रव्य-गुण विज्ञानीय अध्ययन

एल० पी० गुप्ता, एस० पी० सेन एवं के० एन० उडुप

'अर्जुन' का वृक्ष भारत के अधिकांश भागों मे पाया जाता है। आयुर्वेद एवं यूनानी चिकित्सा पद्धति में, यद्यपि इसके छाल का प्रयोग अनेक रोगों में बतलाया गया है, परन्तु दुग्ध के साथ इससे सद्यः निर्मित क्षीरपाक का हृदय रोगों में बहुतायत से व्यवहार देखा जाता है। इसका छाल बाहर से चिकना, भूरा तथा कागज जैसे उखड़ते हुए पर्तों से युक्त होता है। अणुविक्षिकी यंत्र-संरचना में प्रथम कम्ब्रिटेसियस जैसे बाल फिर इपिडर्मिस, कुछ 'कोलेन काइमा' के पर्त तथा एक 'पेरेनकाइमा' का मोटा पर्त अंदर तक प्रविष्ट दीख पड़ता है।

वनस्पतिरासायनिक विश्लेषण में इस छाल के 'पेट्रोलियम-इथर' तथा 'बेन्जोन' अर्कों में स्टेराल (sterol) की उपस्थिति तथा 'क्लोरोफार्म'-अर्क मे 'रिड्यूसिंग सूगर' और 'ग्लूकोसाइड' की अल्प मात्रा एवं 'अलकोहल' अर्क में इन दोनों की अधिक मात्रा पायी गयी।

अलकोहल-अर्क के जलीय-घुलनशील भाग का मेढक के हृदय पर संकोच शक्ति वृद्धिकारक प्रभाव देखा गया। जलीय-घुलनशील भाग को कुत्तों में सिरा-वेध विधि द्वारा देने पर, 'अलिन्द' एवं 'निलय' दोनों के संकोच शक्ति में वृद्धि पायी गयी। इस अर्क के चूर्ण का शशकों में एक मास पर्यन्त मुख द्वारा प्रयोग करने के उपरान्त भी 'प्रोथ्राम्बीन' समय में कोई विशेष परिवर्तन नहीं देखा गया।

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METHODS OF STANDARDISATION OF INDIGENOUS DRUGS

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Introduction

Life and drugs are closely related. In the ancient period people faced the first problem of life that was selection of nutritional substances from the natural resources. The rejection and selection of nutritionally potent substances came across many findings which are closely observed to draw the line of demarcation of toxic and hazardous substances. Later on some of these substances were found to be active. Hence the expedition of natural resources was achieved by experience and not by experiment.

Our country has a vast resources of naturally occuring medicaments as it is evident from the accounts of books like Charaka and Sushruta etc. This very much influenced the western people to find the basis of their approach. In fact the modern medicine has just cemented the bricks placed by the great ancient physicians including Hippocrate and Galen. The idea of development of naturally occuring medicines is to evalute its safe use with maximum activity. This necessitates the scientific approach of the action and maintenance of the uniformity regarding the preparation which is ready for human use. The search for locally available medicaments was initiated long back but systemic study to screen out the unwanted and wanted materials was encouraged during earlier part of 20th century. The scientific investigation of indigenous drugs was started in India at School of Tropical Medicine, Calcutta under the leadership of Chopra *ct al* in 1933 and at CSIR Unit in Ayurveda and Unani College Delhi under Dr. S. Siddiqui (1932). The search of indigenous drugs was further encouraged after the discovery of reserpine the alkaloid from popular plant Sarpagandha.

Although numerous Semisynthetic and Synthetic medicines have taken the role of "majic remedies" but yet indigenous drugs have its own value as a suitable source to find out cheap method of treatment for our vast poor population which depends much on local resources. But at present this study is not restricted to the observations and findings only. Rather a suitable method is to be found out which is accurate enough for the purpose of standardisation of indigenous drugs. This will ensure the therapeutic potentiality of these drugs like that of synthetic drugs.

The word standardisation includes various problems and fields of study. The standardisation includes study from birth of the plant to clinical application. It is a vast study and involves various disciplines. Obviously scientists are looking after isolated parameters but one should have detailed idea of combined

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approach. This review brings about partial success in this types of problem.

The various factors for standardisation of drugs are as follows :—

1. Source of collection and identification,
2. Isolation and standardisation of active constituents &
3. Biological evaluation for activity.

These methods giving rise to primary standardisation and the reference standard is to be made from such study to have optimum activity of the drug and its constituents.

In ancient days the collection and identification of these drugs was mainly done by expert Ayurvedist himself or his reliable disciples. But at present such collection is not possible. The identification and collection is essential factor for indigenous drug standardisation for example *Nardostachys jatamansi* was official drug in Indian Pharmacopoeia but due to lack of uniformity in the preparations it was deleted. Like wise there are numerous other drugs which are not included in the pages of Pharmacopoeia due to such problem. Another big difficulty in identification is the correct nomenclature and its botanical synonyms in different parts of the country. Arora & Arora (1962) reported their difficulties in procuring authentic sample of Gazban (Caccina glauca) for Pharmacological investigation. The maintenance of standard of indigenous medicine has been further uplifted by the attempt of union Ministry of Health to form Ayurvedic and Unani Pharmacopoeia Committee to prepare national formulary and to lay down standards for the control on the manufacture and sale of these medicine.

SOURCE, COLLECTION AND IDENTIFICATION

The cultivation and collection have to be done in the various parts of the country. The percentage of the constituents vary greatly on the climatic condition Thus the localisation of cultivating place serves the useful purpose and also the question of uniform cultivation reduces the variation in activity. Cinchona plant grows better at a relatively high altitude with abundant supply of water. Hence the government has started cultivation of the plant in the Hilly district of West Bengal. Likewise all the medicinal plants like Nuxvomica and Rauwolfia should be cultivated at a place only depending on the habit of the plant. This ensures the uniformity of activity in relation to source.

The collection of drugs should be related to the stage of development and the period of collection. Some work of the vitamin C and tannin content of fresh and dried Amala for different period of storage has been done (Damodaran and Srinavasan, 1935; Mitra and Ghose, 1942; Sastry and Shivaram Krishna, 1942) and therefore a rigid standard for its vitamin and tannin content can be evaluted. Since these active constituents go down on storage, the storage time and collection will have to be specified. Hence further processing of the drug should be done preferably soon after collection unless the stability has been assured. It is desirable to plot a curve of collection period and percentage of active constituents which gives idea regarding best collection season.

The variation of the activity of the crude drugs could be either due to natural abnormality mainly due to condition of cultivation or by virtue of scrupulas practice. Although the procedure of cultivation is within the control of scientific attempt and can be achieved by rational approach, the problem of adulteration cannot be checked up with such simple means. Inspite of this fact the scientific basis has been evaluated as precautionary measure

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and microscopical study of the powder done to control such adulteration.

Microscopical study

The different tissues of the plant are responsible for different function. The histological study reveals the character and arrangement of these tissues as they are present in the drug. Many drugs possess a characteristic structure which are the guidence in identification. Such studies are made either from longitudinal or transverse section or from the powdered drug itself.

CHEMICAL STANDARDISATION AND ISOLATION OF ACTIVE CONSTITUENTS

The chemical standardisation of indian indigenous drugs and preparations is a complicated problem and requires careful scientific investigations. For the purpose of standardisation of these drugs the best approach will be to start the work on individual drugs i. e., the raw drugs first and then the polypharmaceuticals. The method of manufacture is also a very important part of standardisation and therefore the chemical parameters which are used to standardise these drugs should be followed at various stages of these procedures to achieve complete standardisation. The authenticity of drugs plays an important role in these preparations to an extent that it is not only important to have an authentic drug but is almost essential. The standardisation of indigenous drugs on the basis of chemical studies has got its own advantages like exactness of results, quick and less time consuming observations and recovery of the material in cases where the drug to be standardised is in small amounts.

There can be several methods of standardisation of a drug chemically, however, it depends upon the nature of the drug under study that which particular method should be applied. Although no single method can be suggested for standardisation of indigenous drugs there are certain common parameters which can be established. Some of them are as follows :—

1. Extractive values

The drug can be extracted with different solvents like petroleum ether, chloroform, alcohol and water. These extracts can be evaporated to dryness and their constant extractive values with different solvents can be established.

2. Assay of known constituents

The constituent like, total alkaloids, glycosides, tannins, steroids and acid contents etc. can be determined by isolating and estimating the active principles. For examples, Belladona herb and Nux vomica may be standardised for their total alkaloids, Ipecauanha for total non–phenolic alkaloids, Jalap for its resin and Cod liver oil for its vitamin contents. The estimation of active principles of indigenous drug after their isolation in purified form, may be done by using colorimetric, spectrophotometric and spectrophotoflourometric techniques and may be compared with that of standard sample, and by applying ultraviolet, infra-red, nuclear magnetic resonance and mass spectra. The isolation of these active principles can be done by extracting the drug with different solvents with increasing polarity like petroleum ether, chloroform, alcohol and water extracts using the techniques of counter current distribution (Greig, 1962) or solvent extraction and crystallising the materials after obtaining in pure crystalline forms. The techniques of chromatography viz. thin layer, column and gas chromatography can be applied to separate, identify and estimate the active counstitaents present in these drugs. The chromatographic patterns of the various drug

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extracts can be made, their Rf values can be determined and these chromatographic patterns can be used for the future standardisation. Column chromatography using various adsorbants like alumina and silicagel may be very helpfully tried for the separation of the active constituents present in complex mixtures. After separation the constituents can be analysed by applying thin layer chromatography. The analysis of the spots on thin layer plates can be done by either visual means or by applying spectrophotometric techniques. The estimations of drugs can be done directly by spraying with reagent on the plate or after extracting the developed spot with a suitable solvent. The desitometer can be used for the direct estimation of constituents on plates. For example resin and balsoms can be estimated by spraying with ethanolic phosphoric acid (Jork, 1963). Cholesterol (Zollner & Amin, 1962), bile acid (Semennk and Beher, 1966) amino acids (Hara et al, 1964) and lipids (Blank et al, 1964) can be estimated in very small amounts with the help of densitometer. Morphine alkaloids may be estimated by thin layer chromatography up to the range of 60 micrograms (Heusser, 1964). Gas chromatography can also be applied very successfully for the standardisation of indigenous drugs. The gas chromatographic patterns of the various drugs extracts can be prepared by using suitable column. With the gas chromatography it is possible to separate and analyse the most complex mixtures of drugs and determine their qualitative as well as quantitative nature, simultaneously by using one single technique. With recent developments in the simplification of the instrument and the discovery of different type of columns and most sensitive integrators the technique has been very useful for the assay of drugs and their constituents.

The preparative model gas chromatograph is now available which can separate various constituents in a fraction collector alongwith the analysis of the drugs on a paper. The gas chromatography can be used for almost all types of drugs, for example steroids (Vanden Heuvel et al, 1960) vitamins (Zibber et al, 1960) cannabis drugs (Farmilo and Davis, 1961) and amino acids (Gehrike and Stalling, 1967) etc.

The alkaloids, glycosides, sugar and other active principles can also be determined in these drugs by following the methods described in Pharmacopoeia. For Indian indigenous drugs Indian Pharmacopoeia can be taken as a basic book for the estimation of the active constituents. The Indian Pharmacopoeia contains the assay of about eight hundred and fifty drugs and a large number of them are found as a part of indigenous drugs. For examples some of very important drugs like morphine, reserpine, caffeine, quinidine, cocaine, ergotamine, artopine, scopolamine, which are present as the active principles of these indigenous drugs, can be assayed following the methods described in the Indian Pharmacopoeia.

Although nothing can be said of the individual active principles as which one is responsible for the activity, the standard samples of the drug must have nearly constant composition of the active principles. If a drug contains essential oils, it can be steam distilled and the purified sample can be studied for the acid value, ester value, iodine number and other such constants. The glycosides can be determined on weight to weight basis in the drugs. The ascorbic acid contents can be determined by the indophenol titration method.

Some of the indigenous drugs are still to be discovered for their active principles. In these cases it is desirable to investigate them for

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these principles by applying the modern valuable techniques like. infra-red spectra nuclear magnetic resonance spectra, optical rotaroy dispersion and gas chromatography etc.

The sugars can be estimated by a number of reagents like aniline phthalate reagent and Benedicts solution for estimation of sugars. The Benedict solution contains sodium citrate, potasium thiocyanate, sodium carbonate, potassium copper sulphate and potassium ferrocyanide. The solution of the drug containing sugar is titrated with Benedicts qunatitative regent and the amount of sugar, present is calculated by the amount of Benedicts solution consumed for titration.

The pH value of the liquid preparations like asavas, aristhas etc. can be helpful in standardisation of the drugs. The specific gravity of these preparations can also be determined. The liquid preparation can also be seen for their total solid contents.

If a drug is found to contain lipids or fatty materials it can be analysed quantitatively for the component fatty acids by ultra violet alkali isomerisation by argentation and reverse phase chromatography or even gas chromatography. The unusal fatty acids can be detected and determined by infra-red spectra, U. V. spectra and various types of chromatography. The polyunsaturated fatty acids can determined by ozonisation, hydrogenation and oxidative degradation by periodate and lead tetracetate.

Tannin contents of the drugs can be established by precipitating the whole tannin with ferric chloride and weighing the precipitate of ferric tannate or hydrolysing ferric tannate and getting free tannins. This is important as the tannins of a number of drugs like Amla, on hydrolysis gives methyl ester of gallic acid and ellagic acid. The methyl ester of gallic acid has been found to be pharmacologically active and therefore the determination of tannin contents and standardising the drug on this basis will be useful. Similarly the ascorbic acid contents of Amla or other drugs can be determined by colorimetric method. 2, 4–dinitro phenylhydrazine with few drops of concentrated H_2SO_4 gives orange colour with vitamin C. The intensity of this colour at 540 mu can be estimated by comparing with that of standard and the ascorbic acid contents can be estimated. For plant extracts, metaphosphoric acid treatment can be done so as to prevent oxidase enzymes and other interfering compounds. Metaphosphoric acid also acts as a protein precipitant. However, while estimating ascorbic acid, one should have some consideration for the method of storage, preparation etc. of the drug as ascorbic acid content varies with these factors. Like vitamin C, other vitamins could also be assayed.

For the rapid quantitative estimation of various proteinous products, electrophoresis on polyacrylamide gel can be done.

3. Determination of inorganic constituents and ash values

The inorganic constituents of the drugs can be assayed by applying gravimetric and volumetric methods as well as the more sensitive flame photometric method. For determination of the ash contents the dried sample can be ignited on a flame and charred. The ash can be determined separately as water soluble portion with HCl using methyl orange as indicator. The acid soluble portion of the ash and the insoluble portion can also be determined.

The constituents of the ash like iron, aluminium and calcium etc. can be determined by the gravimetric methods. The contents of sodium, potassium, calcium and magnesium in small amount can be estimated with a flame photometer. Calcium and magnesium

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can be estimated on known eliquots of Edta using suitable indicators.

The sulfur containing drugs can be estimated for their sulfur contents in the form of sulphides and sulphates. The chlorides can be estimated by Mohr's method. The carbonates can be estimated by evolving carbondioxide, collecting it is in NaOH solution and treating the solution with barium chloride and finally titrating with an acid.

Work on these lines has been carried out by various workers. Some examples of work carried out in this direction are as follows :

Commiphora mukul (gum guggul) has been standardized by Arora and his co-workers (Arora et al, 1972) in our laboratory and the results obtained are shown in table 1.

Similarly, standardization of a polypharmaceutical drug Chavanprash has been carried out (Arora, 1969) and the results of some of the parameters established in case of three samples of Chavanprash of different Ayurvedic companies are shown in table 2.

Attempts to standardize the indigenous polypharmaceutical preparation Jawarish Jalinoos, used in the treatment of various diseases of the stomach, intestine and urinary bladder was made by Israili, (1971). Six samples from various places were procured and the results of some of the studies are shown in table 3 and 4.

Israili, (1973) has also reported the standardization of Itrifal Mulayyin, a Unani preparation. Seven samples of this drug were analyzed for their specific gravity, pH value, loss of weight on drying, essential oil content, water and alcohol soluble contents, total and acid insoluble ash, total glycosides, reducing and non–reducing sugars, tannin, resin and vitamin contents. The analytical values obtained for these samples were compared with that of a standard reference laboratory sample.

'Kushta Farlad' has also been standardized by Israili, (1972). Various samples of the drug were analysed for their particle size, ascending paper chromatography and by applying spectrophotometry.

Israili and Issar, (1973) have carried out the standardizaion of Myrtus Communis, another Unani drug with the help of pharmacognostic studies. These workers have found that the macroscopic and microscopic studies on the drug are very useful in establishing the morphological and anatomical characters of the drug. They have also found that the chemical analysis of this drug can be utilized for standardization.

BIOLOGICAL ACTIVITY

This is the ultimate mean for determination of the potency of drug in respect of therapeutic activity. Chemically, a drug may contain high percentage of active constituents. This may be biologically less active owing to the presence of different isomers which cannot be differentiated by chemical investigation easily. This guideline is also helpful in searching isomers of different activity like quinine and quinidine. This biological activity of the drug can be estimated in isolated organs or intact preparation and in case of bacteriostatic or bacteriocidal substance the inhibition of the gro vth of suceptible organism is found out. But in all cases the activity is to be measured in comparison with the standard considered as reference drug.

Toxicity studies open a new chapter in the evolution of newer drugs and thus much attention should be paid in it which is more important than the study of beneficial activities. Binnis reported that for the congenital abnormalities in lambs due to plant *Veratrum califor Nicum* (commonly called False Hellebore or Western Hellebore) among the several alkaloids one was responsible for such effect.

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TABLE 1

Standardisation of Commiphora mukul (Gum Gugul)

Extractive values (percent)				Ash value (%)		Thin layer chromatography of metholic extract	
Pet. ether extract	Alc. ext.	Water. ext.	Total	Acid insoluble	Acid solubie	Rf values of 9 spots in the solvent system Hexane : Ether : Acetic acid (96 : 10 : 1)	Rf value of 20 spots in the solvent system Hexane : Chloroform : Acetic acid : Ether acetate : Ether (105 : 20 : 15 : 5 : 5)
13. 25± 0.084.	26. 15± 0.082.	15.90± 0.142	8.15± 0.017	2.45± 0.008	3.58± 0.022	0.0933, 0.16, 0.36, 0.48, 0.5466, 0.60, 0.6933, 0.7533, 0.8666,	0.20, 0.25, 0.03625, 0.4812, 0.7550, 0.6125, 0.6875, 0.7125, 0.8125, 0.9125.

TABLE 2

Standardisation of Chavan Prash

Sample N.o	Extractive value (percent)		Rf values of the cons.		Tanin contents%	Specific gravity
	Alc. Ext.	Water ext.	Alc. Ext.	Water ext.		
1	14.83	12.80	0.89	0.92	6.02	1.44
2	11.14	10.52	0.84	0.86	7.01	1.46
3	21.60	14.67	0.90	0.93	7.5	1.38

TABLE 3

Physico–Chemical Contents for various samples of Jawarish jalinoos

Sample No.	Specific gravity	Wt. on drying%	Essential oil%	Reducing sugar%	Alcoholic soluble content%
Rs	1.349	12.3	0.20	26.7	52.94
A	1.358	13.82	0.13	23.8	53.04
B	1.359	12.25	0.20	19.3	17.12
C	1.356	12.95	0.20	26.6	20.64
D	1.363	21.3	0.20	9.4	25.79
E	1.355	17.76	0.20	5.9	17.6

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TABLE 4

Sample No.	Water soluble matter%	Ph value	Total alkaloids%	Total glycoside as aglysone%	Total ash%	Acid insoluble ash%
Rs	65.17	4.3	0.20	0.27	0.132	0.28
A	65.55	4.3	0.20	0.27	1.023	0.17
B	67.36	4.4	0.18	0.28	1.160	0.11
C	73.74	4.2	0.08	0.28	1.185	0.45
D	62.24	4.6	0.15	0.25	2.805	0.76
E	62.78	3.9	0.12	0.24	0·228	0.00

It is interesting that a small injection of the plant extract given on the fourteenth day of pregnancy would produce teratogenicity in all the animals.

Arora reported that Calophyllolide an active principle fro.n *Calophyllum inophyllum* linn increases the prothrombin time, but after repeated administration the drug did not produce any response. They studied the metabolic fate of 4–phenyl umbeiliferone its methylether and calophyllolide in the rabbits and observed that the increased metabolic rate reduces the activity of the drug. Hence the metabolic study should be carried out as the last parameter to study. This also gives the idea about the toxicity study in particular system.

Summary

The guideline for evaluation and the standardisation of Indigenous drugs has been discussed briefly. The source of collection, identification, isolation of active constituents, characterisation and determination of biological activities are the important parameters for the successful investigation of newer drugs of Indigenous origin. Various methods of study including observation of the toxic manifestations of such drugs are critically discussed in this article.

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OPTIMUM CONDITIONS FOR PRODUCTION OF BERBERINE HYDROCHLORIDE FROM BERBERIS ROOTS (DARUHARIDRA)

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Abstract

A novel process for the production berberine hydrochloride from berberis[1] stem barks and root has been developed. The process is based upon cold percolation of powdered drug with an optimum concentration of aqueous acetic acid (2.5%), addition of hydrochloric acid and cooling to a temperature 2–5°C. The process developed gives a yield of 1.3% from stem bark and 1.9% from roots. It is a significant improvement over the hitherto known process which is based upon extraction of the drug with alcohol and then applying hot conditions for isolation, giving only a yield of 1.0% from the stem bark and 1.3% from the roots.

The optimum conditions were worked out after a series of experimentation.

Materials and Method

Authentic samples of stem bark and roots of *Berberis asiatica* were collected from Bhowali Region of Himalayas. They were disintigrated and uniform samples of 60–80 mesh size were taken for experimentation. Glacial acetic acid (B. D. H.) was used after proper dilutions for percolating the drug. Hydrochloric acid (B. D. H.) was used for addition in the percolate to convert berberine acetate into berberine hydrochloride.

The products obtained from each percolation after addition of hydrochloric acid were cooled in a temperature controlled shelf of a frigidaire (Kelvinator make) at 2° to 5°C.

Samples of powdered berberis stem bark and roots (100g.) were separately percolated with 500 ml. of dilute acetic acid of concentrations : 0.5%, 1.5%, 2.5%, 3.5% and 4.5%. After keeping for 12 hours at room temperature the percolates (I) were drawn off, 5 ml. of hydrochloric acid added and kept in a frige at 2–5° C. The residues of each percolation were repercolated with the same quantity and strength of acetic acid, as previously. The second percolates (II) were again drawn off after 24 hours, and to them 6 ml. of hydrochloric acid added. The product was kept in a frige at 2.5°C. The residues were further percolated with the same quantity and strength of acetic acid. The third percolates (III) were drawn again after keeping for the same

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period (24 hours) and after adding the same quantity of hydrochloric acid (5 ml.) it was kept in a frige at 2–5°C. The residues were rejected after this stage.

Each percolate gave a golden yellow precipitate of berberine hydrochloride which was filtered off and dried in vacuo. After getting the first crop, the second and the third crops were also isolated by keeping the mother liquor in the frige.

Results and Discussion

The results of the recovery of berberine hydrochloride under each set of conditions (both from the stem bark as well as roots) are given as under (table–1 to 5) :

TABLE 1

Extraction of Powdered Berberis Stem Bark and Roots with 0.5% Acetic Acid

	Yield of Berberine hydrochloride	
	Stem bark (g)	Root (g)
Percolate (I)		
Crop 1	0.46	0.67
Crop 2	0.06	0.02
Crop 3	0.00	0.00
Percolate (II)		
Crop 1	0.15	0.22
Crop 2	0.10	0.14
Crop 3	0.04	0.06
Percolate (III)		
Crop 1	0.04	0.06
Crop 2	0.05	0.07
Crop 3	0.00	0.00
Total	0.90	1.30

TABLE 2

Extraction of Powdered Berberis Stem Bark and Roots with 1.5% Acetic Acid

	Yield of Berberine hydrochloride	
	Stem bark (g)	Root (g)
Percolate (I)		
Crop 1	0.56	0.81
Crop 2	0.04	0.06
Crop 3	0.00	0.00
Percolate (II)		
Crop 1	0.17	0.25
Crop 2	0.12	0.17
Crop 3	0.04	0.06
Percolate (III)		
Crop 1	0.04	0.06
Crop 2	0.11	0.16
Crop 3	0.02	0.03
Total :	1.10	1.60

TABLE 3

Extraction of Powdered Berberis Stem Bark and Roots with 2.5% Acetic Acid

	Yields of Berberine hydrochloride	
	Stem bark (g)	Root (g)
Percoiate (I)		
Crop 1	0.62	0.90
Crop 2	0.06	0.08
Crop 3	0.00	0.00
Percolat (II)		
Crop 1	0.22	0.32
Crop 2	0.17	0.25
Crop 3	0.04	0.06
Percolate (III)		
Crop 1	0.04	0.06
Crop 2	0.11	0.16
Crop 3	0.02	0.03
Total :	1.28	1.86

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TABLE 4

Extraction of Powdered Berberis Stem Bark and Roots With 3.5% Acetic Acid

	Yields of Berberine hydrochloride	
	Stem bark (g)	Root (g)
Percolate (I)		
Crop 1	0.62	0.90
Crop 2	0.05	0.07
Crop 3	0 00	0.00
Percolate (II)		
Crop 1	0.22	0.32
Crop 2	0.16	0.23
Crop 3	0.04	0.06
Percolate (III)		
Crop 1	0.04	0.06
Crop 2	0.11	0.16
Crop 3	0.03	0.04
Total :	1.27	1.84

TABLE 5

Extraction of Powdered Berberis Stem Bark and Roots with 4.5% Acetic Acid

	Yield of Berberine hydrochloride	
	Stem bark (g)	Root (g)
Percolate (I)		
Crop 1	0.62	0.90
Crop 2	0.05	0.07
Crop 3	0.00	0.00
Percolate (II)		
Crop 1	0.22	0,32
Crop 2	0.15	0 22
Crop 3	0.04	0.06
Percolate (III)		
Crop 1	0.04	0.06
Crop 2	0.11	0.16
Crop 3	0.03	0.04
Total :	1.26	1.83

A study of the results shows that initially the yields of berberine hydrochloride go on increasing alongwith the increase in concentration of acetic acid used for percolating the drug. The maximum yield is achieved when the drug is extracted with 2.5% acetic acid. Further increase in the concentration of acetic acid does not give higher yields. The yield of berberine hydrochloride remains however statilised even after using higher concentrations of acetic acid like 2.5% and 4.5%.

The yield of berberine hydrochloride was found greater in roots (1.86%) than in the stems (1.28%) under the optimum conditions of extraction with 2.5% acetic acid.

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किलमोड़ा की जड़ से बारबरीन हाइड्रोक्लोराइड बनाने की उपयुक्त अवस्थाएँ

एम० सी० निगम, एस० सी० दत्त, एस० एन० गर्ग एवं एस० पी० एस० दुहन

किलमोड़ा पौधे की जड़ व तने की छाल से बारबरीन हाइड्रोक्लोराइड बनाने की एक सरल विधि निकाली गयी है। यह विधि पौधे की जड़ व तने की छाल से बनाये गये बारीक चूर्ण को पानी एवं एसीटिक एसिड मिश्रण (२.५ प्रतिशत) को रसने की क्रिया द्वारा २.५ डिग्री सेन्ट्रीग्रेड तापक्रम पर ठंढा करने की विधि पर आधारित है। इस विधि से पौधे की जड़ से १.९ प्रतिशत तथा तने की छाल से १.३ प्रतिशत तक बारबरीन हाइड्रोक्लोराइड की मात्रा प्राप्त की जा सकती है। जबकि अभी तक प्रचलित विधि से जिसमें एल्कोहल को गर्म अवस्था के माध्यम में पौधे के चूर्ण को उबालने की क्रिया पर आधारित है, १.३० प्रतिशत पौधे की जड़ से तथा १.०० प्रतिशत पौधे के तने की छाल से प्राप्त होती है। यह विधि अत्यधिक महत्वपूर्ण एवं सरल है। यह विधि पुनरावृत्ति करके तथा निष्कर्ष को भलीभाति पुष्टि करके विकसित की गयी है।

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ELECTRON MICROSCOPIC STUDY ON THE EFFECT OF LIV. 52 ON CARBON TETRACHLORIDE TREATED LIVER

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[Received for Publication on January 9, 1976]

Introduction

The liver is one of the main and largest organ in the body and it usually does not respond to any available medical treatment after reaching a certain stage of degeneration. Moreover most of the drug is metabolised in the Liver after absorption from the Intestines. However, the introduction of Liv. 52, an indigenous compound, has been claimed to be one of the remedies for the treatment of many liver disorders. [1, 3, 4, 6, 7], It has been further reported that it reduces the intrahepatic congestion and cholestasis. The regeneration was induced by this drug was claimed earlier by Sheth *et. al.* 1960.[11] This was later on confirmed by Prasad in 1974, [8, 9] where the cirrhotic liver tissue explants have shown signs of regeneration, when grown in organotype culture, *in vitro,* in the medium containing Liv. 52. According to him it helps also in glycogenesis in addition to the regeneration of liver cell and also decrease fibrotic changes. To find out the action of this drug on liver cell at a subcellular level, this ultra-structure study has been carried out.

Methods and Materials

In this 33 young male mice were selected for study. Out of these, three mice were kept as normal and the remaining 30 mice were divided equally into three groups. In group one all the ten mice were given subcutaneous injection of carbon tetrachloride (in the dose of 0.1 ml. in arachis oil) twice a week upto the end of 60 days. Those animals kept in group two received Liv. 52 drops orally on every alternate day upto 60 days. In addition, they also rceceived subcutaneous injection of carbon tetrachloride in the same dosage and upto the same period as in group one. The remaining animals of group three were given subcutaneous injection of carbon tetrachloride (in the same dose) upto 60 days. Thereafter they received Liv. 52 drops orally on every alternate day for a further 30 days. During this period these animals did not receive the injections o carbon tetrachloride.

Five animals from each group were sacrificed at the end of 30 and 60 days by decapitation. The liver tissue was dissected out and cut into small pieces and fixed immediately in gluteraldehyde solution. After fixing in Osmium tetraoxide the tissue was dehydrated in different grades of alcohol and embedded in Araldite solution. Thcse sections were cut at the thickness of 600 A° by Portar Blum ultramicrotome. All the sections were stained with uranyte acetate and lead nitrate and then

1. Reader.

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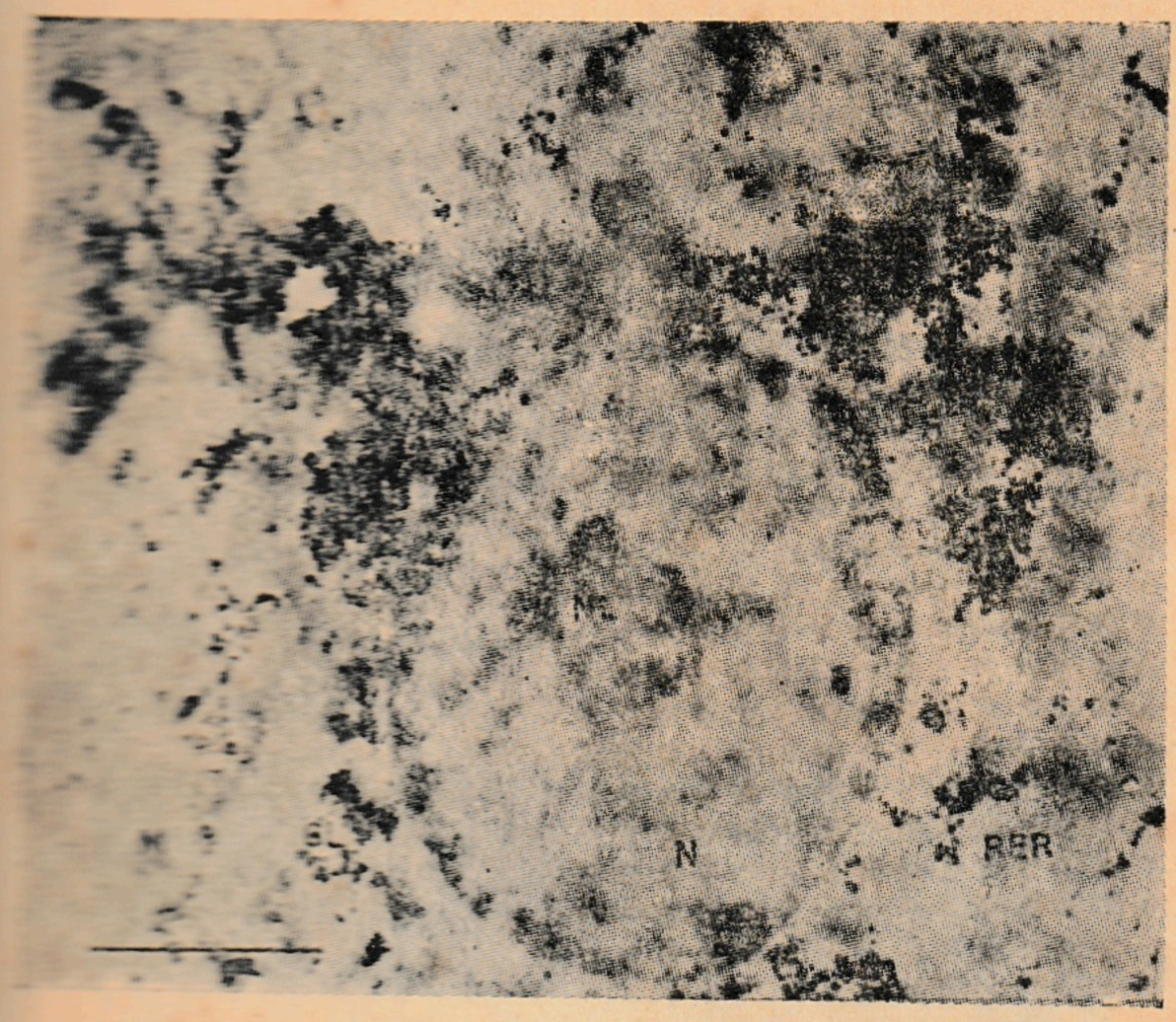

Fig. 1

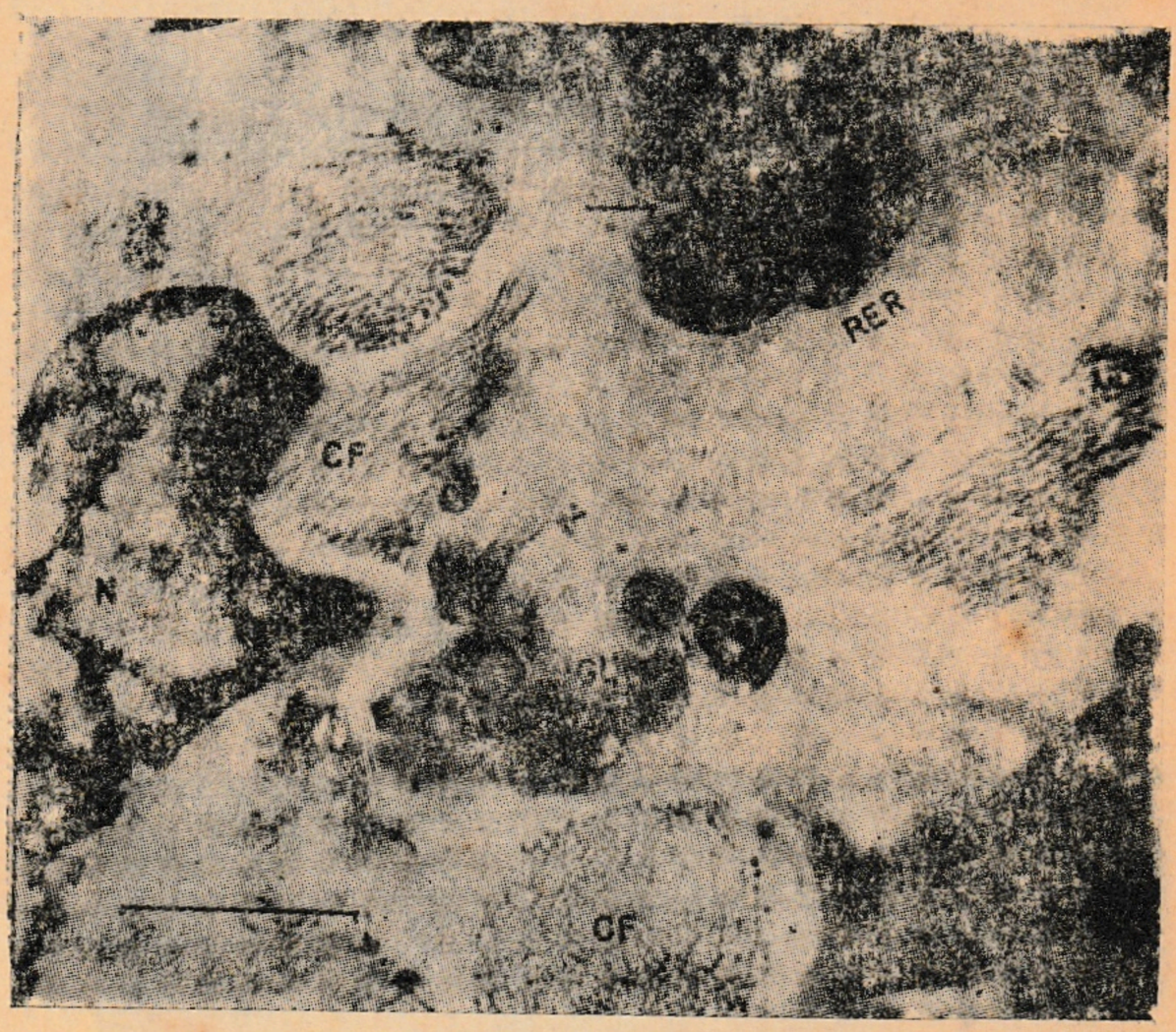

Fig. 2

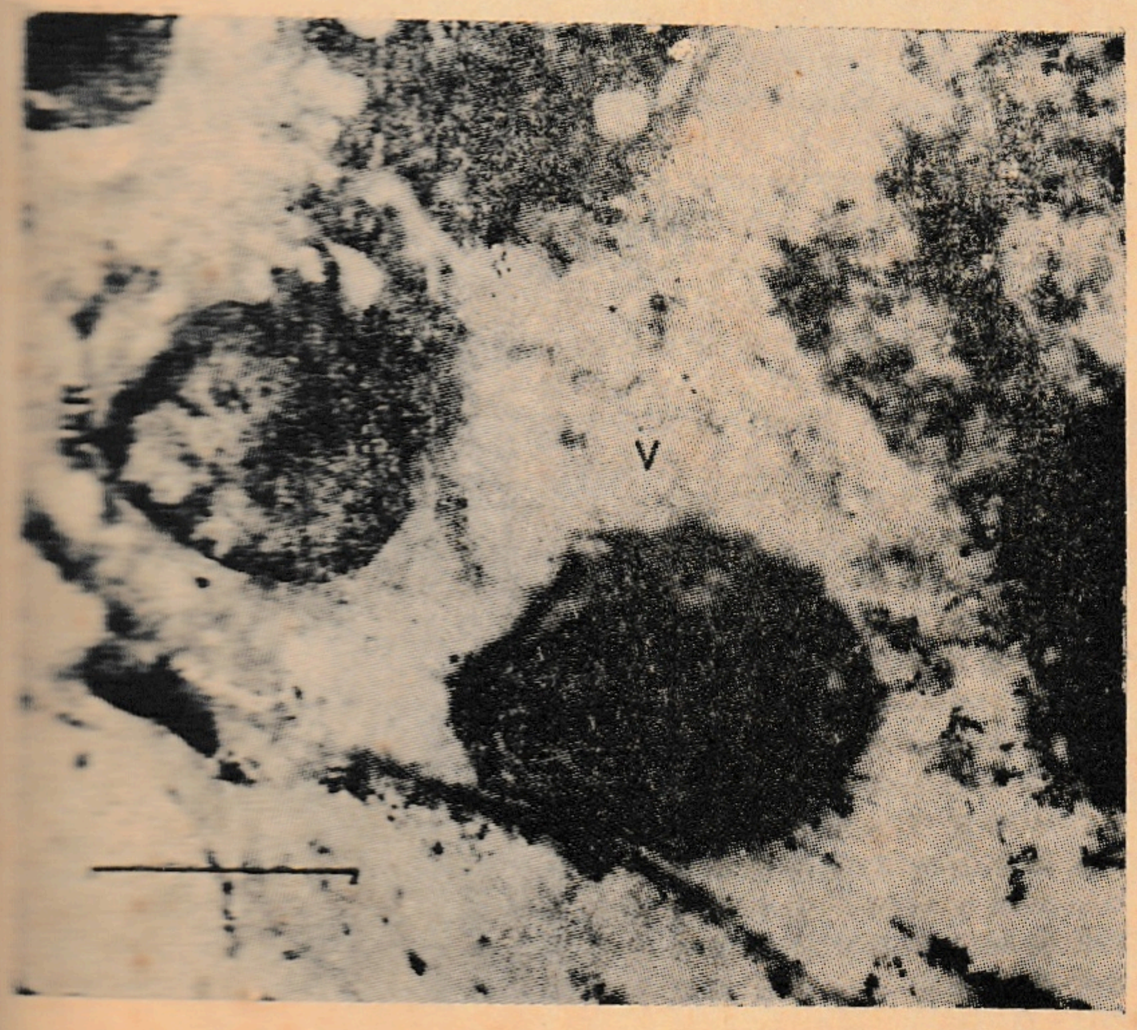

Fig. 3

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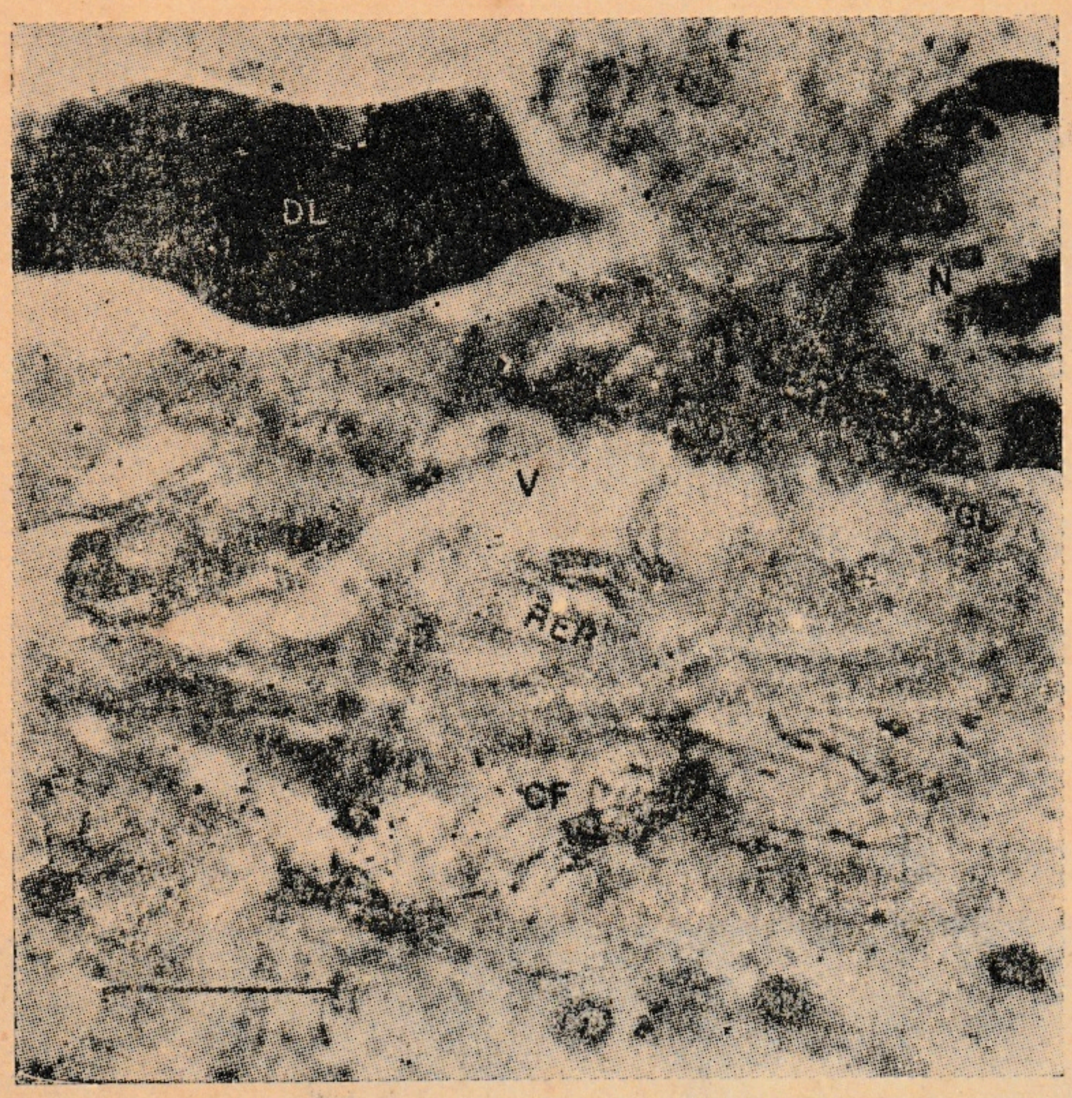

Fig. 4

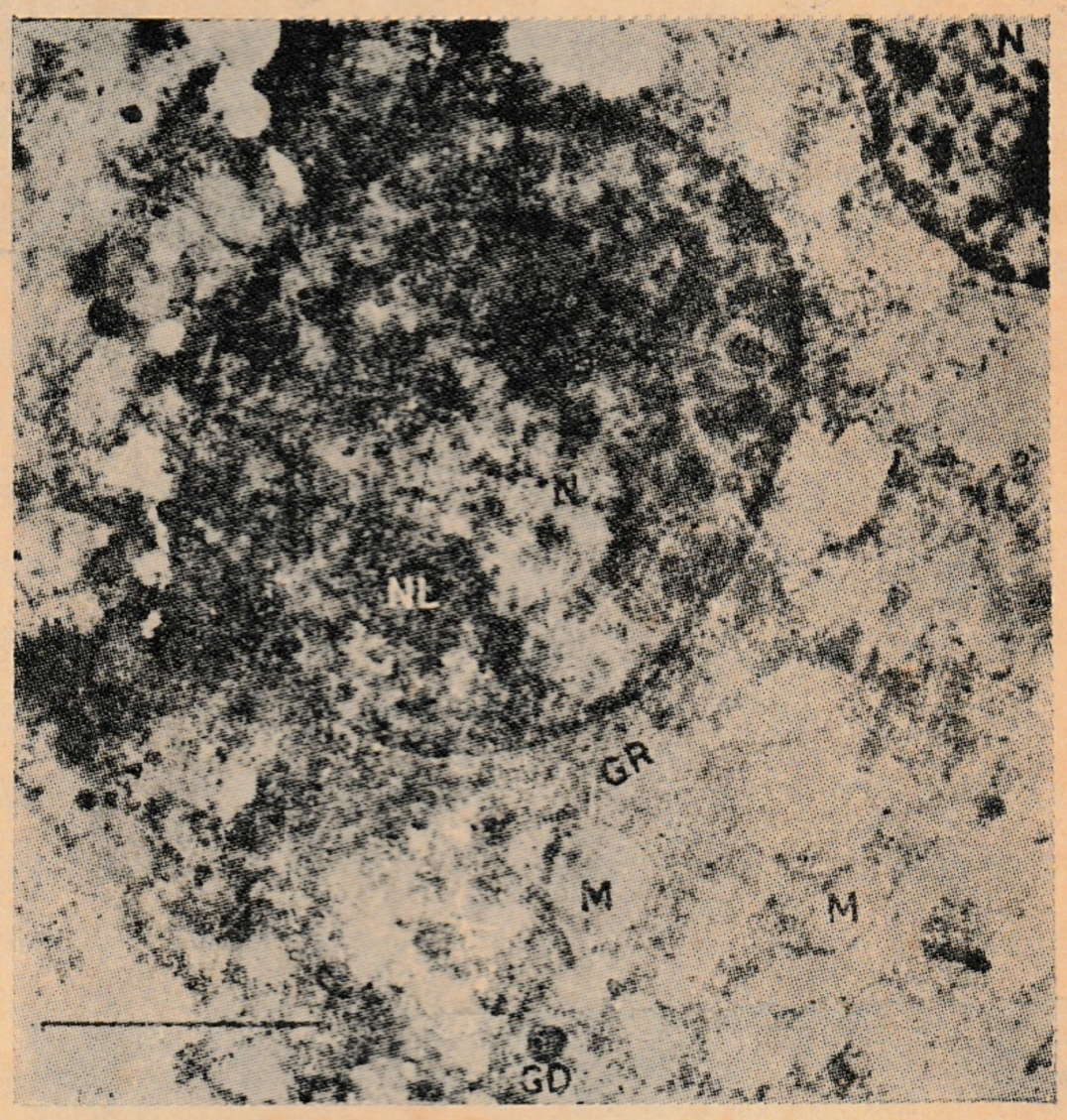

Fig 5

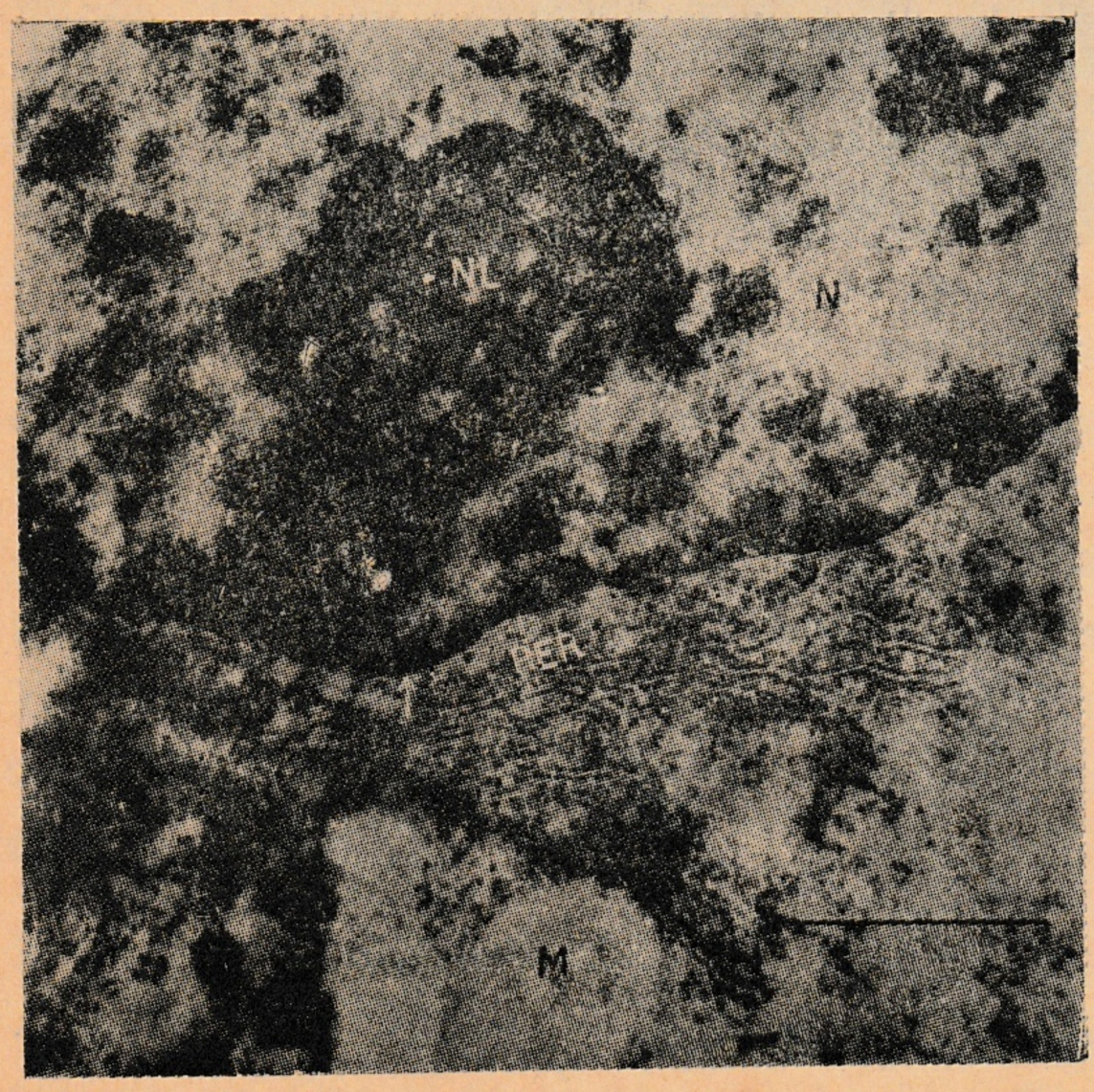

Fig. 6

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examined under Philips Electron microscops 300.

Observations and Discussions

Fig. 1—The Electron microscopy of normal mice liver shows an oval nucleus (N) with nucleolus (NL). The cytoplasm contains a fair amount of glycogen granules of different size (GL). The rough endoplasmic reticulum is (RER) in parallel direction studied with fine grains of Ribosomes. There are fair number of mitochondria (M). X7700.

Fig. 2—The Electron microscopic study of the liver treated with carbon tetrachloride for 60 days shows serrated appearance (indicated with an arrow) of the nucleus (N) with decrease in size. The nucleolus is not well defined. The cytoplasm shows destruction of rough endoplasmic reticulum (RER), which is devoid of ribosomes. Some scattered granular ribosomes coulb be secn suggesting destruction of the nucleic acid i. e. RNA and DNA synthesis. No mitochondria could be located. The glycogen granules have become smaller in size and have clumped like droplets (GL). The cellular membrane is not well defined. Intercellular substance containing fair amount of collagen fibre formation (CF) indicate the stage of fibrotic changes in the liver. X 13000.

Fig. 3—Similar picture showing extensive degenerative changes, the cytoplasm is devoid of its many contents including endoplasmic reticulum with increased number of vacuolation (V), the nucleus has reduced in size. Cell membranes are ill defined. X 7700.

Fig. 4—Those animals treated with carbon tetrachloride along with Liv. 52 show reduction in nuclear (N) serration (indicated with an arrow) with a small nucleolus (NL). The cytoplasm shows reformation of rough endoplasmic reticulum (RER) studded with fine particles of ribosomes. Although the vacuolation still persists (V), fine granules of glycogen have reappeared (GL), The collagen fibre formation (CF) has reduced remarkably. A big secreting droplet (DL) probably billiary secretion could be seen. This suggests that Liv. 52 helps to some extent in neutralizing the action of carbon tetrachloride, stimulating the liver function, and reducing the fibrotic changes in the liver. X 10400

Fig. 5—The Electron microscopic examination of those livers treated with Liv. 52 after the administration of carbon tetrachloride, shows reappearance of nuclear shape (oval) as seen in normal liver (N) with a nucleolus (NL). The serration of the nuclear membrane has disappeared. The cytoplasm contains increased amounts of both smooth and rough endoplasmic reticulum (GR). Mitochondria have started reappearing in little amount of cristal (M). Few secreting droplets (G) can also be seen. X 6300.

Fig. 6—Shows well organised nuclear (N) membrane (indicated with an arrow) with a big nucleolus (NL) suggesting increased Chromotive and nucleic acid synthesis. The rough endoplasmic reticulum (RER) has reappeared and is well organised, studded with dense ribosomes. X 13000. There are increased number of mitochondria (M) which are hypertrophied and are having some organised cristal.

This suggests that the administration of Liv. 52 probably stimulates the liver function not only in its biliary output [5, 10] but also by activating the regeneration of the rough endoplasmic membrane and ribosomes. Thus, it increases the synthesis of Nucleic acid and ribosomal enzymes This confirms many earlier observations both *in vivo* and *in vitro*. Further the liver damaged by carbon tetrachloride and not functioning may also start regenerating and functioning by increasing the

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nucleic acid synthesis. In addition, the glycogenesis has also increased remarkably by the administration of Liv. 52, which corraborates our earlier observation made *in vitro*.

Summary

1. Liver damage was produced in mice by the administration of carbon tetrachloride. In addition, Liv. 52 drops was given orally to these animals along with carbon tetrachloride and also after this treatment.

2. Degenerative and fibrotic changes produced by carbon tetrachloride could be prevented to some extent by the administration of this drug.

3. Further the damaged liver also showed the sign of regeneration of nucleus, endoplasmic reticulum, Mitochondria and also some glycogen granules, when treated with Liv. 52.

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लीव. ५२ नामक औषधिका कार्बनटेट्राक्लोराइड जन्य यकृत् विकृति पर प्रभाव का अध्ययन

जी० सी० प्रसाद

प्रयोगशालीय चूहों में कार्बन टेट्राक्लोराइड द्वारा विकृति उत्पन्न की गई। साथ ही साथ एक वर्ग के चूहों को चिकित्सा के रूप में लीव ५२ भी दिया गया। तीसरे वर्ग में ६० दिन तक केवल कार्बन टेट्राक्लोराइड दिया गया और उसके पश्चात् १ मास तक लीव ५२ द्वारा चिकित्सा की गई। तदनन्तर प्रत्येक वर्ग के चूहों के सूक्ष्म यकृत् खण्डों की एलक्ट्रान माइक्रोस्कोप द्वारा परीक्षा की गई। लीव ५२ द्वारा चिकित्सित वर्ग में विकृति की मात्रा अपेक्षाकृत कम थी और तीसरे वर्ग में जिसमें चिकित्सा बाद में की गई, यकृत् कोशिकावों के पुनरोद्भव के लक्षण प्रतीत हुए।

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ON STANDARDISATION OF ASAVAS/ARISHTA A PRELIMINARY STUDY

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Introduction

Asavas/arishtas form one of the major mediums through which most of the Ayurvedic drugs are administered for various ailments and for rejuvenation. The chemical constituents of the drugs used for their preparation vary according to the age, season of collection and soil conditions. Also, many other factors like the conditions of preparation of arishtas like the time of heating of *kashayas* and *sandhana* etc creep in and as such the fixing up of absolute standards for arishta is very difficult. Hence, it was considered worthwhile to study the effect of heating for different periods of time and using different containers for the preparation of kashayas, on the properties of the arishta and the results are presented in this communication. Amritharishta which is widely used against all types of fever[1] was taken for these studies.

Materials and Methods

The kashayas and arishtas used for experiments were prepared in the laboratory according to the *kashayavidhi* and *asavarishta kalpana*.[2] Most of the drugs were purchased from the local market and a few of them were suppllied by the S. M. P. Units of CCRIMH. These were first pharmacognostically identified and then used for the preparation. In the case of some of the controversial drugs and when the original drugs were not available, the locally used ones were taken for preparation. The list of drugs and the quantity used per yoga of the arishta are given in Table. 1

Fresh stem of Guduchi *(Tinospora cordifolia)* was used; in all other cases, the shade dried drugs were used. These were cut into small pieces and coarsely powdered.

EXPERIMENTS WITH KASHAYA PREPARATION

No definite conditions are prescribed in the Ayurvedic literature regarding the time of heating or the material of the vessel for the preparation of kashaya. It is merely mentioned that the kashaya is to be heated till the volume is reduced to one fourth.[3]

(a) *Effect of heating the kashaya for different duration of time*

One batch of single drugs was divided into four equal parts. The appropriate quantity of water was added and kwatha was heated for 24, 48, 72 and 96 hours respectively. Stainless steel vessels provided with lids were used for the preparation. The flame was

1. Research Officer,
2- Research Officer

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TABLE 1

List of Drugs used for the preparation of Amritharishta
(Ref. Bhaisajyaratnavali, Jvaradhikara)

Sl. No.	Sanskrit Name	Botanical Name	Parts used	Quantity / Unit
1.	Guduchi	Tinospora cordifolia	Fresh Stem	4.800 kg
2.	Bilva	Aegle marmelos	Root	480 gm
3.	Syonaka	Oroxylum indicum	Root	480 gm
4.	Gambhari	Gmelina arborea	Root	480 „
5.	Patala	Stereosperumum suveolens	Root	480 „
6.	Agnimantha	Premna latifolia	Root	480 „
7.	Salaparni	Desmodium gangeticum	Root	480 „
8.	Prsniparni	Uraria picta*	Root	480 „
9.	Brihati	Solanum indicum	Root	480 „
10.	Kantakari	Solanum xanthocarpum	Whole part	480 „
11.	Goksura	Tribulus terrestris	Fruit	480 „
12.	Ajaji	Carum roxburgianum	Seed	768 „
13.	Parpata	Oldenlandia corymbosa	Whole part	96 „
14.	Saptacchada	Alstonia scholaris	Root bark	48 „
15.	Sunti	Zingiber officinalle	Dried rhizome	48 „
16.	Marica	Piper nigrum	Dried fruit	48 „
17.	Pippali	Piper longum	Dried fruit	48 „
18.	Nagakesaram	Mesua ferrea	Flower / Stamens	48 „
19.	Abda (Musta)	Cyperus rotundus	Bulbous root	48 „
20.	Katvi (Katuka)	Picrorrhiza kurrooa	Dried rhizome	48 „
21.	Prativisa (Ativisa)	Aconitum heterophyllum	Dried tuberous root	48 „
22.	Indrayava (Vatsabija)	Holarrhena antidysenterica	Seeds	48 „

Gudam–Jaggery —14.40 kg
Reduced volume —12.29 Litre
Water for decoction —49.18 Litre
Sandhana period —30 days

* We have not been able to get the drug Uraria picta; instead the plant pseudartria vicida, which is used locally for Prasniparni has been used.

adjusted so as to keep the *kwatha* at about 98–100°C (nearly boiling). The vessel was kept opened or partially closed as required so as to have the volume reduced to nearly one fourth in the stipulated period. The volume of the filtered kashaya was then noted and further evaporated to the required volume.

A part of the kashaya was removed which was subjected to chemical analysis and the remaining portion was kept for sandhana in a glazed porcelain vessel after the addition of the appropriate quantities of jaggery and the *prakshepana churna*. All details like the fumigation of the vessel (with ghee–pippali fumes) etc. were observed. After a sandhana period of 30 days, the clears arishtas were obtained, these were then centrifuged at 3000 RPM for 1 hour and subjected to exhaustive

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chemical analysis as laid down in the proforma approved by the Council. Standard analytical procedures were followed.[4] Results of the analysis of *kashayas* and of *arishtas* obtained from these are presented in Tables 2 and 3.

The organoleptic characteristics of the *arishtas* were tested by the Ayurvedic staff of the Circuit as well as by some of the eminent Ayurvedists in Trivandrum.

(b) *Effect of material of vessel used for kashaya preparation*

Many of the practising physicians of Kerala are of the view that only seasoned earthenware are to be used for the preparation of kashayas. The effect of using vessels made of aluminium, stainless steel and freshly tinned copper on the properties of the kashaya and arishta obtained from them are hitherto unknown and hence it was considered worthwhile to investigate this aspect.

Four batches of kashayas were prepared from the same list of drugs using stainless steel, aluminium, freshly tinned copper and seasoned earthenware respectively. But for this difference, all other conditions of preparation and sandhana of the arishta were the same. The kashaya was analysed and the results are given in Table 4. The arishtas obtained after a sandhana period of 30 days from these were then subjected to the routine chemical analysis and their organoleptic properties were tested. The results are given in Table 5.

Results and Discussion

Typical results are presented in Table 2 to 5.

It is found that maximum quantity of materials is extracted by 96 hours extraction. This is evident from the values of specific gravity, total solids, tannins, protein content

TABLE 2

Analytical data of the Kashayas prepared for different duration of Heating

Sl. No.		Duration of heating			
		24 hrs	48 hrs	72 hrs	96 hrs
1.	Specific gravity	1.016	1.016	1.016	1.019
2.	pH	4.7	5.0	4.9	4.9
3.	Total solids (g/100 ml.)	3.25	3.5	3.71	4.2
4.	Tannins (mg/100 ml.)	20	225	250	300
5.	Ash (g/100 ml.)	0.518	0.550	0.650	0.650
	(a) Acid soluble (g/100 ml.)	0.385	0.410	0.490	0.470
	(b) Acid insolube (g/100 ml.)	0.130	0.140	0.160	0.180
6.	Alcohol precipitation				
	(a) Proteins (mg./100 ml.)	53.2	86.6	93.32	173.32
7.	Ether extractables (mg./100 ml.)	55.6	72.8	90.4	107.8
	(a) Acidic (Carboxylic + Phenolic)	18.2	23.0	33.4	42.9
	(b) Basic	nil	nil	nil	nil
	(c) Neutral	31.6	40.6	45.7	49.6

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TABLE 3

Analytical Data of Arishtas from Kashayas of different duration of heattng (Vessel for Sandhana : Glazed porcelain)

Sl. No		Duration of heating			
		24 hrs	48 hrs	72 hrs	96 hrs
1.	Specific gravity	1.247	1.238	1.244	1.216
2.	pH	4.1	4.3	4.7	4.8
3.	Total solids (g/100 ml)	64.8	63.3	64.16	58.97
4.	(a) Total sugar (g/100 ml)	57.52	55.28	54.0	50.4
	(b) Reducing sugar (g/100 ml)	46.2	43.88	40.88	38.28
	(c) Non–reducing sugar (g/100 ml)	11.72	11.4	13.12	12.12
5.	Alcohol (%)	2.54	2.54	2.54	3.14
6.	Titratable acid (≡ ml 0.1N NaOH)	53.6	50.4	46.4	44.0
7.	Volatile acid (≡ ml 0.1N NaOH)	3.84	5.12	3.84	5.12
8.	Ash	1.135	1.134	1.14	1.115
	(a) Acid insoluble ash	0.144	0.158	0.148	0.145
9.	Total tannins (mg/100 ml)	625	650	675	700
10.	Alcohol precipitation				
	(a) Proteins (mg/100 ml)	300	370	380	410
	(b) Carbohydrates (g/100 ml)	1.25	1.27	1.87	1.87
11.	Ether extractable:				
	Total (mg/100 ml)	131.3	144	155	163
	(a) Carboxylic (mg/100 ml)	49.7	50	50.1	55.1
	(b) Phenolic (mg/100 ml)	12.3	12.5	15.3	18.3
	(c) Basic (mg/100 ml)	nil	nil	nil	nil
	(d) Neutral (mg/100 ml)	28.3	29.0	33.3	38.8

TABLE 4

Analysis of Kashaya Prepared using different Vessels

Sl. No.		Vessel for kashaya preparation			
		Alumin–ium	Stain–less steel	Tinned copper	Earthen–ware
1.	Specific gravity	1.014	1.014	1.015	1.013
2.	Total solids (g/100 ml)	3.2	3.15	3.66	3.10
3.	Tannins (mg/100 ml)	212	212	237	212
4.	Ash	0.469	0.513	0.563	0.499
5.	Qualitative analysis of ash	Al^{++} Fe^{+}	Fe^{+}	Fe^{+}	Al^{+} Fe^{+}

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TABLE 5

Analytical Data of Arishtas prepared from Kashayas obtained by using Different Vessels

(Vessel used for Sandhana—Glazed porcelain jar)

Sl. N0	Vessels for kashaya preparation			
	Alumi-nium	Tinned copper	Earth-enware	Stainless steel
1. Specific gravity	1.215	1.227	1.229	1.212
2. pH	4.7	4.8	4.4	4.6
3. Total solids (g/100 ml)	58.6	62.0	63.0	57.7
4. Total sugar (g/100 ml)	45.6	46.04	47.12	44.56
5. Reducing sugar (g/100 ml)	32.83	30.02	33.28	32.60
6. Non-reducing sugar (g/100 ml)	12.72	16.06	13.84	11.96
7. Alcohol (%)	3.14	3.14	3.14	3.14
8. Titratable acid ($\equiv$ 0.1N NaOH)	54.1	48.5	89.7	58.2
9. Volatile acids ($\equiv$ 0.1N NaOH)	3.23	2.58	6.46	3.23
10 Ash (%)	1.82	1.95	2.01	1 9
11. Acid insoluble ash (%)	0.200	0.180	0.160	0.160
12. Tannins (mg/100 ml)	520	588	493	493
13. Alcohol precipitation				
(a) Proteins (mg/100 ml)	310	330	260	275
(b) Carbohydrates (mg/100 ml)	0.75	1.25	0.55	0.675
14. Ether extractables (mg/100 ml)	224	273	267	226
(a) Acids	60	55	68	65
(b) Phenolic	34	47	45	34
(c) Basic	nil	nil	nil	nil
(d) Neutral	68	105	77	65

and ether extractables. Further increase in the duration of kashaya preparation was not attempted, since the general practice in Ayurveda is to use a maximum of four days for kashaya preparation.

There does not appear to be much variation in the analytical values. Iron is present in all the kashaya samples. The kashaya prepared by using earthen vessel and aluminium vessel contain appreciable amounts of aluminium. Tinned–copper vessel would appear to be the most suitable, in view of the fact that tin is comparatively inert to the chemical constituents of the kashaya.

A perusal of Table 5 shows that there is no appreciable difference in the analytical values of the arishtas obtained from kashayas prepared in vessels made of different materials viz. stainless steel, tinned copper, aluminium and earthenware. However, from the practical point of view, stainless steel appears to be the best.

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Summary

In the present study, some experiments have been carried out on the effect of duration of heating and the nature of the vessel on the physical, chemical and organoleptic properties of Amritharishta. It has been found that 96 hours of heating gives maximum extractable materials in the preparation of Amritharishta. These seems to be no appreciable change with the change of material of the vessel during the arishta preparation.

ACKNOWLEDGEMENT

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Jour. Res. Ind. Med. Yoga & Homoeo. 11 : 4, 1976.

EFFECT OF PULSATILLA NIGRA, A HOMOEOPATHIC DRUG ON THE UTERI AND ESTRUS CYCLES IN ALBINO RATS

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Introduction

Studies on the action of gonadal hormones on the uterus of mammals is extensive as has been brought out by Galand *et al.* (971) on mice. They discerned that 0. 3, 1. 2, and 10.0 microgram estradiol had almost similar action on the uterus but doses higher than 10 microgram brought about sloughing off and proliferation of uterine epithelia. while Perrotta (1962, 1966) found fluctuations in the rate of the mitotic divisions in the uterinc epithelium of rats after oestradiol administration, Eooker and Forbes (1947) discerned progesterone to increase the number of the stromal cells in the endometrium of rats. They detected number and cellular enlargement also-Clark (1973) noticed an inhibition in the usual response to oestradiol by the epithelial cells in rats pretreated with progesterone, on the other hand the uterine stroma exhibited the mitotic response. Martin *et al.* (1973) indicated that progesterone to suppress DNA synthesis in the glandular as well as luminal epithelium of the uterus of mice in the presence or absence of oestrogen.

Reuter *et al.* (1970) cbserved in the golden hamster progesterone delayed ovulation. Further, he found oestrus behaviour was dependent upon the dose, the vehicle used and time of administration. Gilmore and Mc Donald (1969) discerned in rats progesterone given on the third day shortened the estrous cycles to four days, whereas, estrogen minimised this period to three days. Inspite of the existence of abundant data in this area, Laumas *et al.* (1974) have stated that "Not much is known about the precise mode of action of progestational steroids and more so about the parent compound, progesterone.

Earlier work caerried out in this laboratory (Chandrasekhar and Rao, 1972, 1973, a.b; Chandrasekhar and Sharma, 1972) has proved the efficacy of the homoeopathic drug *caulophyllum* as an anti–implantation and antiovulatory agent. Their studies also indicated that the drug acted via the hypothalamo–hypophysialgonadal axis (Chandrasekhar and Rao, 1974 unpublished). The earlier findings gave the necessary impetus to take up the present work using another homoeopathic drug namely *Pulsatilla*. Work deals with the drug's effect on the uterus and oestrus cycles in the rat.

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Materials and Methods

About 100 female albino rats, weighing 110 ± 10 gms., and 95 to 100 days of age were used. The rats were inbred in the laboratory and were housed under normal conditions of photoperiod. They were fed balanced laboratory diet and water was allowed *ad-libitum*. After 25 days of acclimation, the estrus cycles were determined by vaginal smears technique. Under this photoperiod, shedule most of the rats exhibited a five and half day estrus cycles with the diestrus period being the longest. Thus rats with regular five and half days estrus cycles were selected and divided into 3 groups. The experimentation was started when the rats were in the proestrus phase.

GROUP I

Control—These rats were orally administered 0.3 ml. each of 90% alcohol, which constitutes the base of the drug, for 3 successive days.

GROUP II

Experimental—These rats were orally administered 0.3 ml. each of 30 potency of Pulsatilla for 3 successive days.

GROUP III

Experimental—These rats were orally administered 0.3 ml. each of 200 potency of Pulsatilla for 3 successive days.

Initial administration of drug was done immediately after the onset of proestrus and thereafter at 24 hours intervals. From each group the uteri of 5 rats were fixed first after 12 hours and thereafter on days 1, 2, 3 and 5. They were weighed prior to fixing after removing all extraneous tissue. Histological examination of the uteri was done by staining the 5 micro thick sections in Haematoxylin and Eosin. Micro-histo-morphometric methods were employed to assess the results by ocular and stage micrometer caliberations (Siddique and Chandrasekhar, 1972).

Note—30 & 200 Potencies of Pulsatilla—One part of the mother tincture when triturated with 9 parts of alcohol, it forms first decimal potency, when it is attenuated with 99 parts, it forms the first centesimal potency. In this experimentation the 30 and 200 Potencies of Pulsatilla used were purchased from Messers Hahnemann publishing Co. Private Ltd. Calcutta (India).

Results

TABLE 1

Change in uterine weight mg/100 gm. body weight.

	12 hrs	1 day	2 days	3 days	5 days
Control	222.5±15.9	163.3±29.0	164.5±14.4	125.5±15.1	70.5±2.5
30 Potency	180.6±6.5	166.0±26.0	144.0±16.0	119.0±2.8	170.0±28.7
200 Potency	175.0±7.9	164.0±20.3	90.0±10.0	92.6±5.4	183.0±19.7

TABLE 2

Variation in Endometrial height in micra.

	12 hrs.	1 day	2 days	3 days	5 days
Control	335.0±41.9	335.0±54.5	333.8±31.9	326.3±25.0	282.5±5.0
30 Potency	371.9±50.6	278.1±37.0	280.6±8.1	286.9±9.5	371.9±85.1
200 Potency	375.0±16.3	303.8±32.0	295.0±20.0	240.0±6.9	375.0±31.8

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TABLE 3

Variation in height of luminal epithelial cells of uterus in micra.

	12 hrs.	day 1	days 2	days 3	days 5
Control	44.5±2.5	49.6±8.3	38.4±3.6	27.3±0.5	23.3±2.8
30 Potency	38.9±1.2	46.0±0.5	32.64±0.5	25.8±2.8	30.9±8.7
200 Potency	29.75±0.2	26.4±0.2	32.5±1.2	23.8±1.7	40.1±4.1

ALTERATION IN ESTRUS CYCLES

Following administration of the drug Pulsatilla (30 and 200 Potency) there was a marked change in the duration and sequence of the normal estrus cycles. In the control groups the estrus cycle was of five and half days duration (proestrus to diestrus) and in 30 potency treated group in about 40% cases the duration between proestrus and diestrus was reduced to 4 days. On the other hand in the 200 potency administered groups the cycle was of 4 days uniformly in all cases as indicated in the table 4.

CHANGE EXHIBITED BY THE UTERUS

Change in weight as well as the histological changes exhibited by the endometrium and the luminal epithelium of the uterus are shown in tables 1 to 3. Changes discerned in the endometrial glands and the mitotic figures in the glandular and luminal epithelia as well as the stromal cells at various stages are described below.

T. S. of Uterus—12 hours : The glandular and luminal epithelia of control (Fig. 1) showed mitotic divisions whereas in 30 potency drug treated case (Fig. 2) mitotic divisions were discernible only in the glandular epithelium. On the other hand in 200 potency drug administered group (Fig. 3a and b) the mitotic divisions were seen neither in the glandular nor in the luminal epithelium. In 200 potency group, stromal cells were comparatively larger than in the other two cases. Similarly the endometrial glands of the 200 potency drug treated rats (Fig. 3b) were more elongated and more secretory than those of the control and the 30 potency treated cases.

One day—Luminal epithelium of the control (Fig. 4) had more cells showing mitotic divisions than in the luminal epithelium of 30 potency treated case (Fig. 5a). On the other hand the epithelium of 200 potency drug treated case (Fig, 6) was highly reduced, had corrugation and the cells showed no mitotic divisions. On day 1, the endometrial glands of 30 potency drug treated group (Fig. 5b) were more elongated and secretory.

On day 2, in the controls the luminal epithelium still showed hypertrophy and mitotic divisions, with multinucleated masses (Fig. 7).

TABLE 4

	Initial	12 hrs.	day 1	day 2	day 3	day 4	day 5
Control	P	P	E	Late E	M	D	D
30 Potency	P	E	E	M	D	P 40%	E 40%
						D 60%	D 60%
200 Potency	P	E	E	M	D	P	E

P = Proestrus E = Estrus M = Metestrus D = Diestrus

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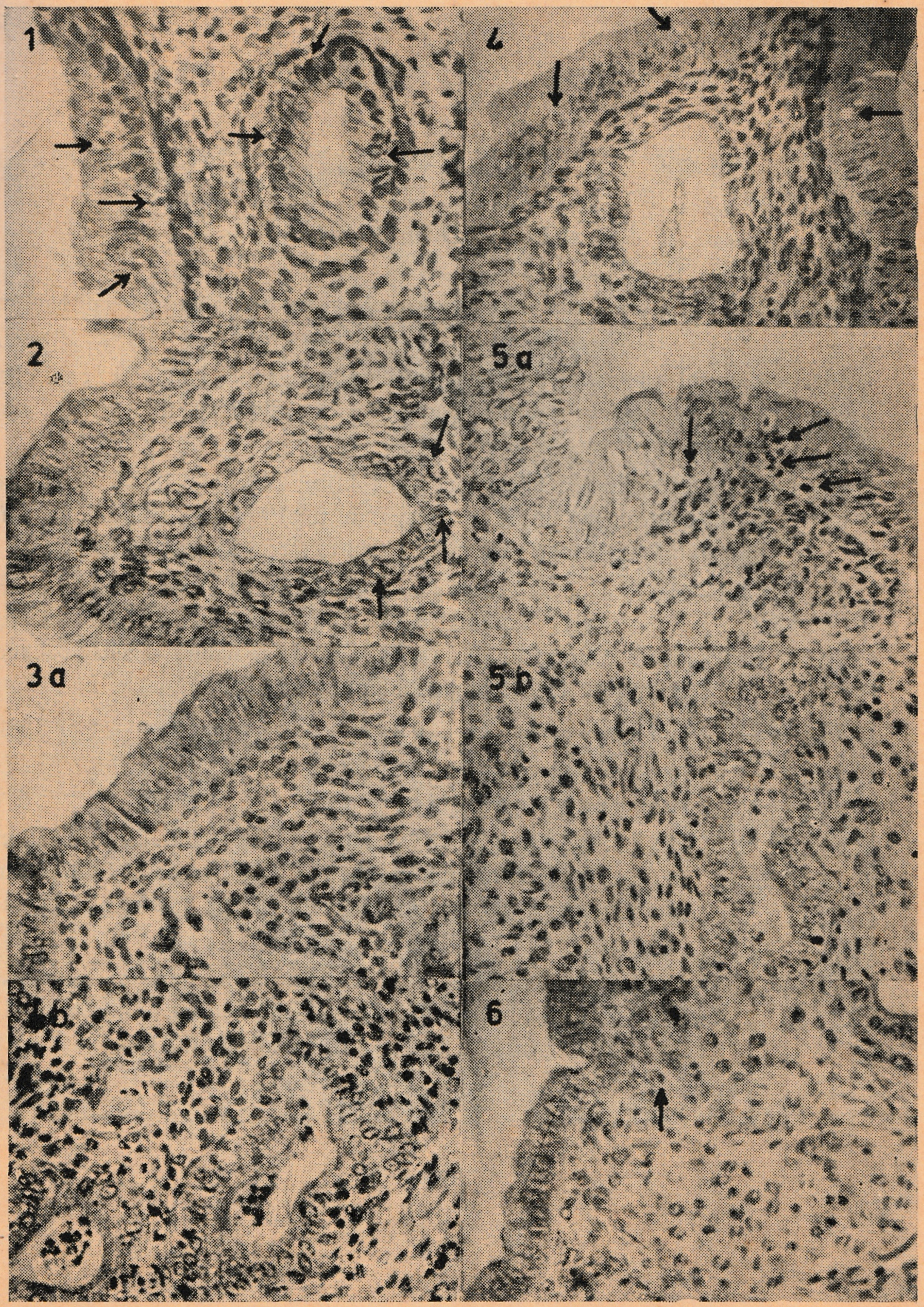

PLATE. 1

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LEGEND OF PLATE 1

Fig. 1–3—T. S. of 12 hours uterus showing Luminal and glandular epithelium. (H. E. 10×40).

Fig. 1—Control shows hypertrophy of luminal and glandular epithelia; swollen nuclei; mitotic figures (arrows indicate the same) and separation of small stromals cells.

Fig. 2—30 potency treated shows mucosal folding; stromal cells not separated as in control; no mitotic figures in the luminal epithelium, and glandular epithelium with mitotic figures (arrows indicate the same).

Fig. 3 a & b—200 potency treated shows luminal and glandular epithelium respectively. Luminal epithelium corrugated; glands dilated and secretory; stromal cells with large nuclei densely packed; no mitotic figures in glandular and luminal epithelia.

Fig. 4–6—T. S. of 1 day uterus (H. E. 0×40).

Fig. 4—Control shows hypertrophy of luminal epithelium with mitotic figures; small stromalcells are densely packed.

Fig. 5a & b—30 potency treated–shows luminal and glandular epithelium respeetively; corrugated epithelium with mucosal folding and mitotic figures (arrows indicate the same); gland dilated and secretory (56).

Fig. 6—200 potency treated–shows luminal and a portion of glandular epithelium reduced epithelium with corrugation; stromal cells hypertrophy having mitotic figures (arrow indicate the same).

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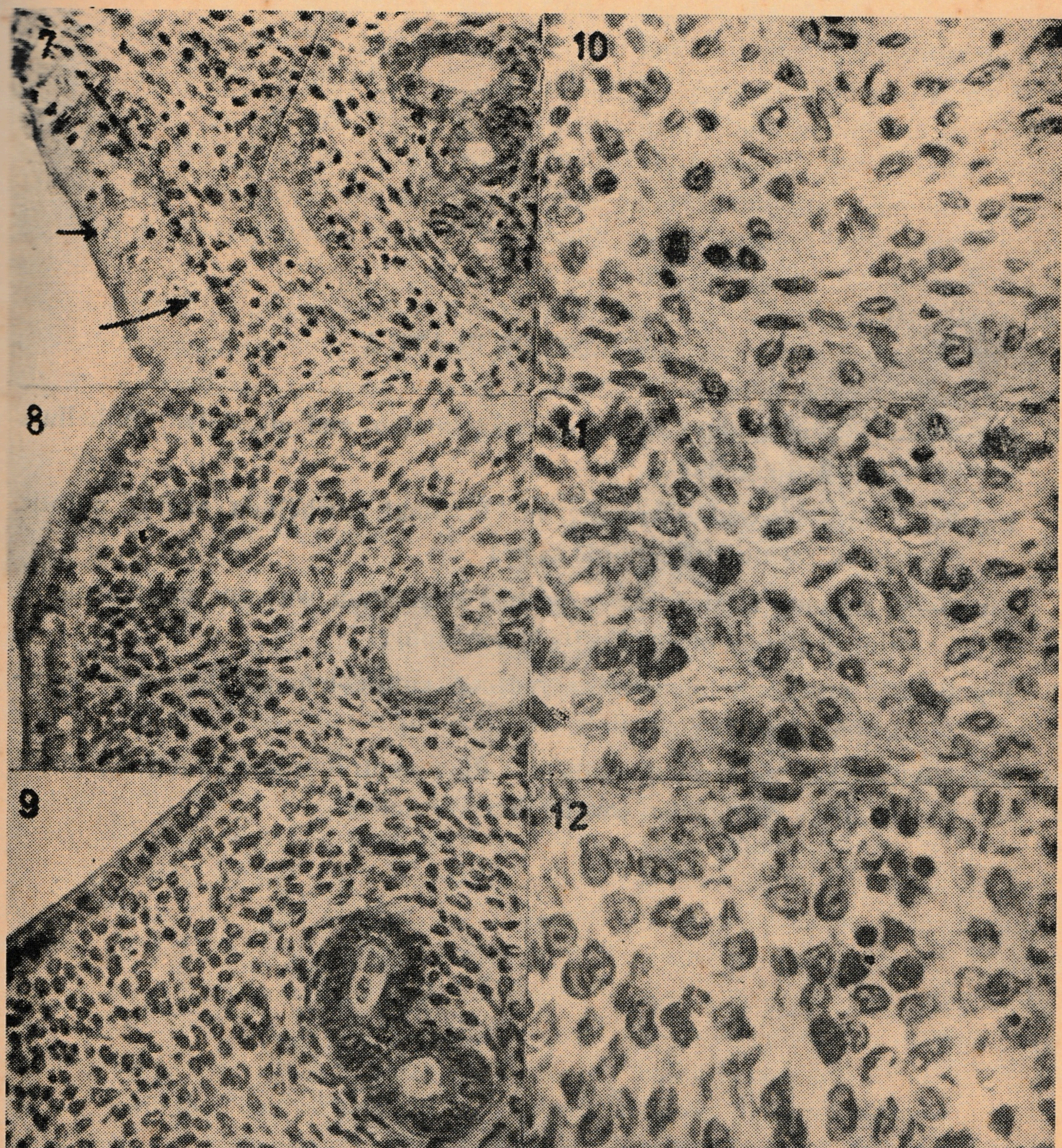

PLATE. 2

P. T. O.

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LEGEND OF PLATE 2

Fig 7–9—T. S. of 2 days uterus showing luminal and glandular epithelium (H. E. 10 × 40).

Fig. 7—Control shows-hypertrophy of lumilnal epithelium having mitotic figures (arrowsindicate the same).

Fig. 8—30 potency treated–shows reduced epithelium with no mitotic figures; dilated glands; stromal cells hypertrophy.

Fig. 9—200 potency treated–shows reduced epithelium; stromal cells hypertrophy; secretory glands.

Fig. 10–12—Showing stromal cells of 2 days control 2 days 30 potency and 2 days 200 potency treated uteri respectively.

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Whereas the 30 (Fig. 8) and 200 (Fig. 9) potencies drug administered groups had highly reduced epithelium which did not exhibit mitotic divisions. The stromal cells of 30 and 200 potency were hypertrophied when compared to the controls (Fig. 10, 11 and 12).

After day 3, the uteri of 200 potency drug treated rats showed significant decrease in weight ($p < 0.05$). Decrease in the endometrial ($p < 0.001$) and luminal epithelial cell heights ($p < 0.05$) in this group was evident (Tables 2 and 3).

5 days—The experimental groups exhibited noticeable increase in uterine weight, endometrial and endothelial cell heights increased over those of the controls (Tables 1, 2 and 3).

Discussion

The results (Tables 1 to 3) indicate that Pulsatilla 30 and 200 potencies inhibited the action of endogenous estrogen. But withdrawal of the drug resulted in an early manifestation of estrous behaviour and increase in uterine weight. These findings are in conformity with the findings of Brown–Grant in rats 1967, Reuter, *et al.* (1970) in Golden hamster and Zimbelman and Smith (1966 a and b); Roussel, *et al.* (1969); Boyd, (1970) in beef and dairy cattle, who used various doses of progesterone in different vehicles at different periods and found early manifestation of estrous cycle. In the control and experimental groups after day 1, there is no marked change in uterine weight. This may be because of highest estrogen titer present at the time of estrus as per the suggestions of Turner and Bagnara (1971). The histological pictures are confirmatory of this argument because in the control the mitotic figures in the luminal as well as in the glandular epithelia of 12 hours, and days 1, 2 treated animals are higher than the experimental animals. It is so in view of the fact that the controls were in their proestrus, estrus and late estrus periods when the estrogen titer was highest. On the other hand in 30 potency *Pulsatilla* treated cases mitotic figures were seen in the glandular epithelium at 12 hours and in luminal epithelium on day one, whereas 200 potency of *Pulsatilla* inhibited the mitotic figures both in luminal and glandular epithelia at all stages. As far as the mitotic figures in the stromal cells are concerned there were no mitotic figures in the controls but a few were discernible in the 30 and more in the 200 potency treated cases. These findings are in agreement with the observations of Clark (1971) who showed that pretreatment with progesterone inhibited the epithelial mitotic divisions caused by estrogen and mitotic divisions were almost entirely restricted to the region of the stroma. According to the present findings there is slight reduction in the luminal epithelial cell height in 30 potency and more in the 200 potency group. This shows that the drug is antioestrogenic. At the same time, there is hypertrophy of stromal cells to some extent in the 30 potency and more in the 200 potency Case, which is more marked on the day 2. These findings are in the conformity with those of Everett (1962) who showed *in vitro* that progesterogenic medium stimulates the increase in size and number of stomal cells and dilation of uterine glands were discernible in the treated groups. Precocious secretory activity of the endometrial gland in the 200 potency cases showed that the drug acts synergistically with normal levels of estrogen. A similar reaction is discernible in the 30 potency cases but only after 24 hours. While in the controls the secretion was meagre.

Martin *et al.* (1973) attributed corrugation of the uterine epithelium to progestational activity. In this investigation (Figs. 3a and 6) which prove beyond doubt the progesterone

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like properties of the drug, specially the 200 potency.

Summary

Two potencies of the drug *Pulsatilla nigra,* namely 30 and 200 reduced the duration of estrus phase and the withdrawal of the drug on the 4th day brought back the proestrus phase. 30 potency of the drug showed this only in 40% of the cases while 200 brought about the result in all the cases.

A remarkable suppression in the uterine weight was noticed in the 200 potency group as long as the treatment was continued. Reduction in the endometrial height and endothelial cell height was found to be greater in the 200 potency group and lesser in the 30 potency group when compared to the controls. Suppression of mitotic divisions in glandular and luminal epithelia was evident in the 200 potency treated group, whereas it was less marked in the 30 potency drug administered case. Endothelia exhibited corrugations in the experimental groups but not in the controls. These changes lead to the conclusion that the drug is 'anti–estrogenic'. Perhaps the 200 potency possesses 'progestogenic' property also because it brought about hypertrophy of the stromal cells which was less marked in the 30 potency treated group and not noticeable in the control group.

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हिन्दी सारांश

पल्सटीलानाइग्रा का इस्ट्रस साइकिल एवं गर्भाशय पर प्रभाव

के० चन्द्रशेखर, सुरेश प्रसाद एवं सी० वी० राव

पल्सटीलानाइग्रा नामक होम्योपैथिक औषधि के दो विभिन्न शक्तियों के घोल का अध्ययन प्रयोगशालीय प्राणियों में किया गया। इनके प्रयोग से इस्ट्रस फेज़ की अवधि घट जाती है और इसके बन्द करने पर पुनः पूर्व इस्ट्रसफेज़ लौट आता है। जहाँ तक दो शक्तिवाले घोलों का प्रश्न है ३० पोटेन्सी की अपेक्षा २०० पोटेन्सीवाले योग में अधिक प्रभाव देखा गया। इसके प्रयोग से गर्भाशय की श्लैष्मिक कला पर भी काफी प्रभाव देखा गया।

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EFFECT OF MILD TO MODERATE EXERCISE ON LIPID FRACTIONS

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Introduction

Serum lipids are known to alter under a number of situations, like type and time of food intake and various mental and physical stresses. We have been interested in the effect of some of the active principles of Gum Guggulu *(Commiphora mukul)* on the lipid pattern of serum.

Since hospitalization means change in physical, mental and dietary habits, we prefer to study the lipids while the subjects are doing their normal work at home and office and are taking the type of food that they are normally accustomed to. Therefore, the subjects have to reach hospital from different distances using different modes of transport including walking from their residences. This involves mild to moderate physical exertion.

We were therefore interested to find if mild to moderate exercise has any effect on the lipid pattern of the serum. We report here our results obtained after replicate measurements on the serum lipids before and after moderate exercise.

Material

1. Only male volunteers consisting of staff members of the Department of Medicine were selected for this study

2. Their age range was from 24 to 46 years with median aga of 30 years.

Methods

1. Fasting blood was collected after half an hour of rest in an air-conditioned room in the morning.

2. Exercise consisted of climbing four times up and down, a flight of four stories of the hospital consisting of 69 steps. This exercise was completed in 8-10 minutes. This was again followed by half an hour rest period and blood samples were redrawn.

3. Replicate measurements on the following lipid compartment were performed each time.

(a) Lipoproteins by the Method of Davis and Ornstein,[4] –Modified by Ananth Narayanan[1],[2].

(b) Free Fatty Acids by the Method of Anstall and Trujellow[3]

(c) Triflycerides by the Method of Felix G. Soloni[5]

(d) Total Cholesterol by the Method of MacIntyre and Mary Ralstion (Introduced by E. J. King)[6]

(e) Total Lipids by the Method of Pernokis, Freeland and Kraus[7],[8]

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TABLE 1

Effect of Exercise (Mild to Moderate) on Lipid Levels

No.	Subject Age (in yrs)	Body Wt. in Kg.	Cholesterol mg% Before Exercise	Cholesterol mg% After Exercise	Triglycerides mg% Before Exercise	Triglycerides mg% After Exercise	F. F. A. Acid mμ% Lit Before Exercise	F. F. A. Acid mμ% Lit After Exercise	Total Lipids mg% Before Exercise	Total Lipids mg% After Exercise	Lipo Proteins% Alpha B	Alpha A	Beta B	Beta A	Prebeta B	Prebeta A
S.	24	58	159	175	41	40	1074	790	490	530	31	62	69	38	–	–
B.	46	68	298	313	147	138	1550	1410	1200	1120	42	40	58	60	–	–
C.P.	24	57	152	140	100	85	1280	1375	900	940	31	45	50	37	19	18
C.	27	65	108	112	58	42	1750	1330	710	730	31	19	69	60	–	21
R.K.	30	84	223	204	200	178	1533	1710	1250	1210	28	36	51	44	21	20
M.D.	40	55	146	138	43	42	900	1210	910	820	64	49	21	30	15	21
D.	32	57	169	162	43	44	1433	1333	800	780	60	74	40	26	–	–
M.	29	65	207	200	90	80	1410	1500	920	950	40	35	60	65	–	–
C.	30	58	247	243	77	57	1727	1336	1050	1010	78	39	22	44	–	17
H.L.	45	62	257	266	57	57	1710	1120	930	1000	52	32	26	68	22	–
C.	29	65	211	207	50	40	1363	909	910	830	49	59	24	41	27	–
M.	29	60	235	255	112	106	1300	1233	1010	1030	55	44	34	56	11	–
ADS.	32	54	255	246	112	106	1000	1033	750	730	61	60	39	24	–	16

B = Before exercise A = After exercise

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TABLE 2

Statistical Analysis

Lipids (n = 13)	Before Exercise Mean ± S. D.	After Exercise Mean ± S. D.
Total Cholesterol (Mg%)	205 ± 54.8	205 ± 58.6
Triglycerides (Mg%)	87 ± 47.4	78 ± 43.5
Free Fatty Acid mμ/lit.	1387 ± 274.9	1253 ± 246.6
Total lipids (Mg%)	910 ± 200.8	898 ± 184.4
Lipo Proteins (%)		
Alpha (α)	48 ± 15.6	46 ± 14.8
Beta (β)	43 ± 17.5	46 ± 14.9

Results

Results were subjected to Wilcoxon matched–pairs signed–Ranks test to find the significance of the effect of exercise on lipid levels.

There was significant reduction ($P < .01$) only in tryglycerides after exercise but not in other lipid levels. In all cases $P > 05$.

Summary

It was found that mild to moderate exercise has no appreciable effect upon the lipid patterns, except for triglycerides which are significantly reduced.

ACKNOWLEDGEMENT

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हिन्दी सारांश

सामान्य व्यायाम का लिपिड-अवयवों पर प्रभाव

डी० बी० बिस्ट तथा जे० पी० कोटियाल

स्वस्थ व्यक्तियों के एक समूह में सामान्य व्यायाम का प्रभाव सीरम लिपिड्स के विभिन्न अवयवों (components) पर किया गया। अध्ययन से ज्ञात हुआ कि सामान्य व्यायाम से सीरम ट्राईग्लिसराइड में महत्त्वपूर्ण कमी आ जाती है, लेकिन सीरम टोटल लिपिड्स, सीरम फ्री फैट्टी एसिड्स, सीरम कोलिस्ट्रोल, लाइपो प्रोटीन्स में कोई महत्त्वपूर्ण परिवर्तन नहीं होता।

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Jour. Res. Ind. Med. Yoga & Homoeo. 11 : 4, 197c.

SURVEY OF EDIBLE PLANTS FOR CALCIUM OXALATE CRYSTALS–I

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(Received for publication on March 8,1975)

Introduction

The edible plants are endowed with capacity to synthesize a wide variety of chemical substances, some of which when ingested by man or animals are known to exert an immediate or cumulative deleterious effect. Some plants have high level of oxalic acid which has the ability to bind the calcium, thus affecting the free calcium availability to the system and so become toxic. Oxalic acid is otherwise non toxic but for the reactions of the oxalate ions. Oxalic acid occurs in the plants in the form of soluble or insoluble oxalates. Kingsbury (1964) has reported that larger concentrations of soluble oxalates result in the precipitation of oxalate crystals in the kidney tubules in the form of small rhomboidal or rosette crystals completely occluding the lumen of the tubule. In severe cases, the lumena of almost all tubules are plugged producing symptoms similar to those of renal insufficiency. Necrosis of epithelial cells occurs when the oxalate crystals come in contact with them. Calculi may be formed in the urinary tract. Fasett (1966) on the other hand critically evaluated the literature pertaining to this problem and concluded that manifestation of chronic effect of food stuff containing calcium oxalate crystals will require rather impossible combination of circumstances, namely a very high intake of oxalate containing food alongwith simultaneous low calcium and vitamin D intake over a prolonged period.

It was, however, considered worthwhile to have a survey of edible plants for the presence of calcium oxalate crystals which might help the dieticians in prescribing desirable vegetables and fruits for those who have the tendency to form stones in digestive or renal systems. Calcium oxalate crystals often form a character of considerable diagnostic importance as they occur abundantly in plant tissues and vary widely in size and appearance from species to species.

The present communication deals with some of the common vegetables indicating presence or absence of calcium oxalate crystals, their type and also distribution in the plant tissues of the concerned species. The important features are given in Table I and illustrated in Plate I from Figs. 1 to 20.

Material and Method

Samples of vegetables were procured fresh from the market as well as from the vegetable gardens. Transverse sections of the edible parts were examined microscopically. The types and distribution of crystals in specific tissues were observed and recorded. The sections were treated separately with nitric, acetic and hydrochloric acids for 5–10 minutes, mounted in glycerin and observed microscopi-

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TABLE 1

Root and stem vegetables

Vernacular name	Botanical name	Family	Edible parts	Frequency of crystals	Types of crystals	Tissue in which crystals are present
Bhasinda or Kamal kakri (H)	*Nelumbium nucifera* Gaertn.	Nymphaeaceae	Rhizome	—	—	—
Shalgom (H) Turnip (E)	*Brassica rapa* Linn.	Cruciferae	Root	—	—	—
Muli (H) Radish (E)	*Raphanus sativus* Linn.	,,	Leaf, root	++	Prismatic	Parenchyma
Ganth-gobi (H) Kohl rabi (E)	*Brassica gongyloides* Linn. var. *caulorapa* DC.	,,	Short stem	—	—	—
Band-Gobhi (H) Cabbage (E)	*Brassica oleracea* var. *capitata* Linn.	,,	Leaves	—	—	—
Alu (H) Potato (E)	*Solanum tuberosum* Linn.	Solanaceae	Tuber	—	—	Cortical and pith parenchyma
Shakarkand (H) Sweet potato (E)	*Ipomoea batatas* Lamk.	Convolvulaceae	Root	+++	Rosette	
Gajar (H) Carrot (E)	*Daucus carota* Linn. var. *sativa* DC.	Umbelliferae	Root	—	—	
Pyaj (H) Onion (E)	*Allium cepa* Linn.	Liliaceae	Bulb	—	—	—
Lasun (H) Garlic (E)	*Allium sativa* Linn.	,,	Clove	—	—	—
Arvi (H) Taro (E)	*Colocasia esculenta* (L.) Schott.	Araceae	Corm	+++	Acicular	Parenchyma
Suran	*Amorphophallus campanulatus* (Roxb.) Bl.	Araceae	Corm	+++	Raphides	Parenchyma
Adrak (H) Ginger (E)	*Zingiber officinalis* Rosc.	Zingiberaceae	Rhizome	—	—	—
Amada Mango ginger	*Curcuma amada* Roxb.	,,	,,	++	Prismatic	Parenchyma

Continued

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Leaf vegetables							
Curry leaf	(E)	*Murraya koenigii* Spreng.		Leaf	++	Prismatic	Midrib parenchyma
Kulpha	(H)	*Portulaca oleracea* Linn.	Portula-	Leaves	+++	Rosette	Bundle sheath
Purslane	(E)		caceae	Stems		—	cortex, pith
Sarson	(H)	*Brassica campestris* Linn.	Cruciferae	„	+	Prismatic	Midrib cortex,
Mustard	(E)	Var. *sarson* Prain					pith
Methi	(H)	*Trigonella foenumgraecum*	Legumi-	Leaf,	++	Prismatic	Mesophyll
Fenugreak	(E)	Lour.	nosae	Stem	++		Parenchyma
Hingcha	(B)	*Enhydra fluctuans* Lour.	Compo-	Leaf,	—	—	—
			sitae	stem			
Lettuce	(E)	*Lactuca sativa* Linn.	„	Leaves	—	—	—
Dhaniya	(H)	*Coriandrum sativum* Linn.	Umbelli-	Leaves	—	—	—
Coriander	(E)		ferae				
Soya	(H)	*Anethum sowa* Kurz	„	Leaves,	—	Prismatic	Cortical
				stem	+		Parenchyma
Chaulai	(H)	*Amaranthus viridis* Linn.	Amaran-	Leaf,	+++	Rosette	Spongy parenchyma
			thaceae	stem	+++	clustered	Parenchyma
Marsa	(H)	*Amaranthus lividus* Linn.	„	Stem	+++	Clustered	Parenchyma
Bathua	(H)	*Chenopodium album* Linn.	Chenopo-	Leaf,	+++	Rosette	Midrib parenchyma
			diaceae	stem	+++	—	Pith parenchyma
Vilayati Palak	(H)	*Beta vulgaris* Linn.	„	Leaf, stem	+++	Clustered	Parenchyma
Chard beet	(E)	var. *cicla* Moq.			+++		
Palak	(H)	*Spinacia oleracea* Linn.	„	Leaf	+++	Rosette	Parenchyma
Spinach	(E)						
Poi Indian	(H)	*Basella rubra* Linn.	Basellaceae	Leaf, stem	++	Rosette,	Palisade tissue
Spinach	(E)				++	prismatic	Cortex
Flower and Fruit vegetables							
Phulgobi	(H)	*Brassica oleracea* var.	Cruciferae	Flower	—	—	—
Cauliflower	(E)	*botrytis*					
Bhindi	(H)	*Abelmoschus esculentus*	Malvaceae	Fruit	+	Rosette	Mesocarp
Okra	(E)	(L) Moench					parenchyma

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Matar	(H)	*Pisum sativum* Linn.	Leguminosae	Fruit	—	—	—
Peas	(E)			Seeds			
Sem	(H)	*Dolichos lablab* Linn.	,,	Fruit	—	—	—
Beans	(E)	var. *typicus* Prain		Seeds			
Palwal	(H)	*Trichosanthes dioica* Roxb.	Cucurbitaceae	Fruits	—	—	—
				Leaves			
Lauki	(H)	*Lagenaria siceraria* (Mol.) Standley	,,	Fruit	—	—	—
Bottlegourd	(E)			Stem			
Kaddu	(H)	*Cucurbita moschata* Duchense	,,	Fruit	—	—	—
Pumpkin	(E)			Stem			
Kundru	(H)	*Coccinia grandis* (L) Voigt syn. *C. indica* W. & A.	,,	Fruit	+	Prismatic	Mesocarp Parenchyma
Karela	(H)	*Momordica charantia* Linn.	Cucurbitaceae	Fruit	+++	Prismatic	Mesocarp Parenchyma
African Cucumber	(E)						
Baigan	(H)	*Solanum melongena* Linn.	Solanaceae	Fruit	+	Prismatic	—
Brinjal	(E)						
Tamatar	(H)	*Lycopersicon esculentum* Mill.	,,	Fruit	++	Prismatic	Pulp
Tomato	(E)						
Mirch	(H)	*Capsicum annuum* Linn. var. *acuminatum* Fingh	,,	Fruit	—	—	—
Pepper	(E)						
Papita	(H)	*Carica papaya* Linn.	Caricaceae	Fruit	+	Prismatic	Parenchyma
Papaya	(E)						
Katahal	(H)	*Artocarpus heterophyllus* Lam.	Moraceae	Fruit	++	—	—
Jackfruit	(E)						
Amla	(R)	*Emblica officinalis* Gaertn.	Euphorbiaceae	Fruit	++	Acicular	Mesocarp
Myrobalan	(E)						
Sahajan	(H)	*Moringa oieifera* Lam.	Moringaceae	Fruit	—	—	—
				Leaf	++	Rosette	Midrib

—, absent; +, rare; ++, frequent; +++, abundant; H, Hindi; E, English.

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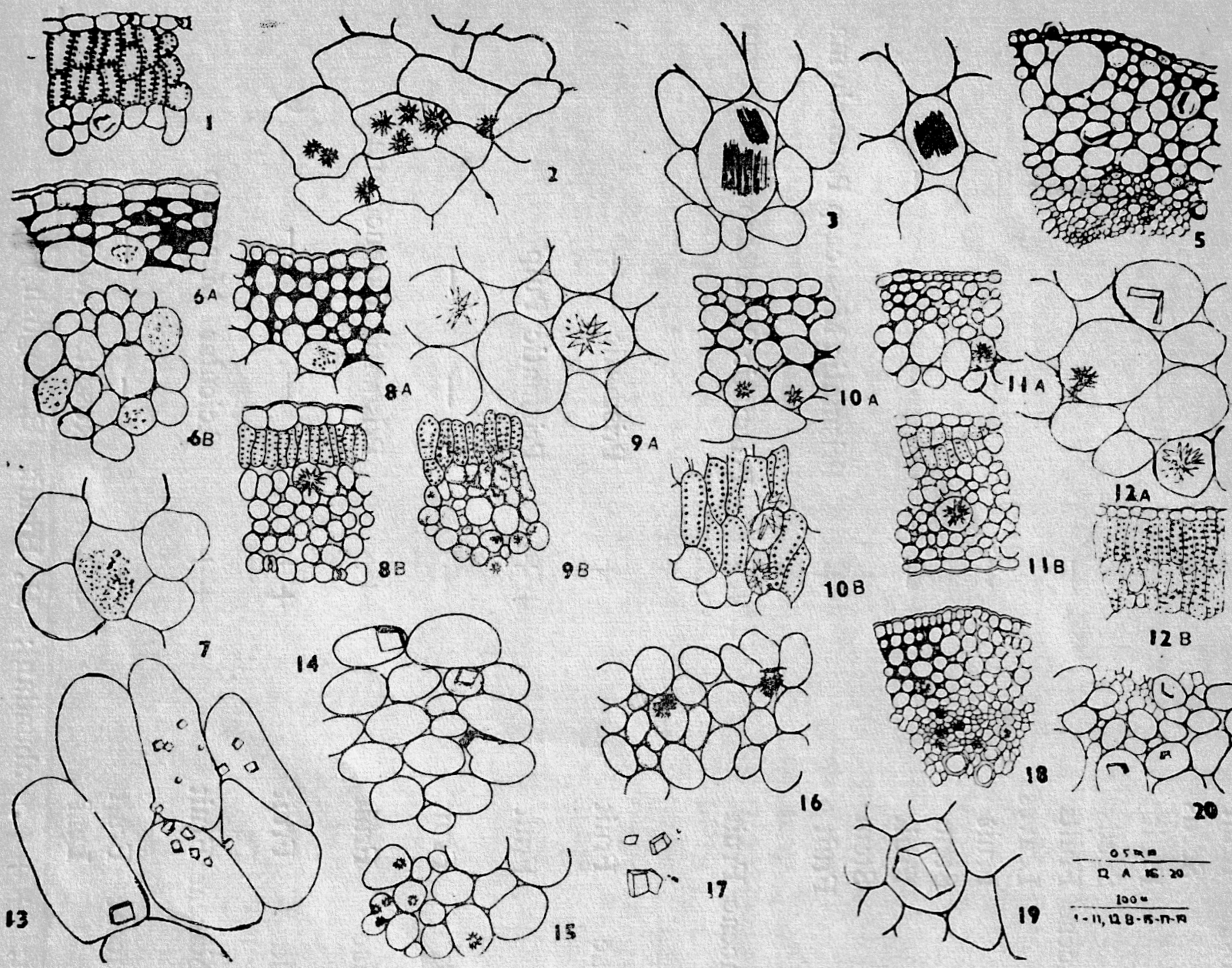

PLATE I

Figs. 1–20—Crystals in different tissues of edible plant parts of various species as seen in transverse section.

1—Prismatic crystal in mesophyll of *Raphanus sativus* Linn. leaf.

2—Rosette crystals in parenchyma of *Ipomoea batatas* Lamk. tuber.

3–4—Needle shaped crystals in parenchyma of *Colocasia esculenta* (L.) Schott and *Amorphophallus ccmponulatus* (Roxb.) Bl. corm.

5—Prismatic crystals in midrib of *Murraya koenigii* Spreng. leaf.

6A–B—Clustered crystals in cortex and pith of *Amaranthus lividus* Linn. stem.

7—Clustered crystals in mesophyll of *Beta vulgaris* (L.) var. *cicla* Moq. leaf.

8A–B—Clustered and rosette crystals in parenchyma of *Amaranthus viridis* Linn. stem and leaf.

9A–B—Rosette crystals in parenchyma of *Portulaca oleracea* Linn. stem and leaf.

10A–B—Rosette crystals in parenchyma of *Chenopodium album* Linn. stem and leaf.

11A–B—Rosette crystals in mesophyll of *Spinacia oleracea* Linn. petiole and lamina.

12A–B—Prismatic and rosette crystals in parenchyma of *Basella rubra* Linn. stem and leaf.

13—Prismatic crystals in mesocarp of *Momordica charantia* Linn fruit.

14—Prismatic crystals in parenchyma of *Coccinia grandis* (L) voigt fruit. ←

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cally. The solubility of the crystals in the nitric or hydrochloric acids and insolubility in acetic acid confirmed the presence of calcium oxalate crystals in different vegetables.

Discussion

Four types of crystals, viz., prismatic, rosette, clustered and acicular have been found present in the vegetable species which have been studied in the present investigation. It has been observed that although the presence or absence of crystals is not indicative of any particular family yet in vegetables belonging to families Araceae, Amaranthaceae, Basellaceae and Chenopodiaceae, acicular and clustered crystals are invariably present.

Among the vegetables in which root or stem is the edible part, *Ipomoea batatas* (Shakarkand), *Colocasia esculentus* (Arvi) and *Amorphophallus campanulatus* (Suran) have abundant crystals, rosette type in the first named and acicular in the last two plants. According to Kirtikar (1896) some of the cultivated variety of 'Suran' when taken internally cause darting pain in the mouth giving the feeling of piercing sharp needles. The crystals with sharp needle–like ends found in the cellular tissue were accounted for their irritant properties.

Moderate amount of prismatic crystals have been observed in the roots of *Raphanus sativus* (Muli) and stem of *Curcuma amada* (Amada). The root and stem vegetables, such as *Nelumbium nucifera* (Bhasinda), *Brassica rapa* (Shalgam), *Daucus carota* var. *sativa* (Gajar), *Brassica gongyloides* var. *caulorapa* (Ganthgobhi), *Brassica oleracea* var. *capitata* (Bandhgobhi), *Solanum tuberosum* (Alu), *Zingiber officinalis* (Adrak), *Allium cepa* (Piyaj) and *Allium sativa* (Lahsun) do not possess any crystals.

Among the leafy vegetables, abundant rosette and clustered crystals have been noted in *Portulaca oleracea* (Kulpha), *Amaranthus viridis* (Chaulai), *A. lividus* (Marsa), *Chenopodium album* (Bathua), *Beta vulgaris* var. *cicla* (Vilayati Palak) and *Spinacia oleracea* (Palak). Of the leafy vegetables which have only moderate amount of crystals, mention may be made of *Trigonella foenum–graecum* (Methi) and *Murraya koenigii* (Curry leaf) (both having prismatic crystals), *Moringa oleifera* (Shahajan) and *Basella rubra* (Poi) (both having rosette crystals). The leaves of *Enhydra fluctuans* (Hingeha), *Lactuca sativa* (Lettuce), *Coriandarum sativum* (Dhaniya), *Anethum sowa* (Soya), *Brassica oleracea* var. *botrytis* (Phulgobhi), *Pisum sativum* (Matar), *Trichosanthes dioica* (Palwal), *Lagenaria siceraria* (Lauki) and *Cucurbita moschata* (Kaddu) do not contain any crystals at all.

The flower and fruit vegetables worked out in the present study have shown abundant prismatic crystals in *Momordica charantia* (Karela) and *Lycopersicon esculentum* (Tamatar). Moderate amount of rosette crystals are present in *Artocarpus heterophyllus* (Katahal) and acicular ones in *Emblica officinalis* (Amla). Crystals are rarely present in the

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→15—Rosette crystals in parenchyma of *Artocarpus heterophyilus* Lam. fruit.

16—Needle shaped crystals arranged like a feather in parenchyma of *Emblica officinalis* Gaertn. fruit.

17—Prismatic crystals in pulp of *Lycopersicon esculentum* Mill. fruit.

18—Rosette crystals in midrib of *Moringa oleifera* Lam. leaf.

19—Prismatic crystal in cortical parenchyma of *Trigonella foenumgraecum* Linn. petiole.

20—Prismatic crystals in pith region of *Curcuma amada* Roxb. rhizome.

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unripe fruit of *Carica papaya* (Papita), *Solanum melongena* (Baigan), *Coccinia grandis* (Kundru) and *Hibiscus esculentus* (Bhindi); if present these have been found to be of prismatic type, The flowers of *Brassica oleracea* var. *botrytis* (Phulgobhi) fruits of *Pisum sativum* (Matar), *Dolichos lablab var typicus* (Sem), *Trichosanthoes dioica* (Palwal), *Lagenaria siceraria* (Lauki), *Cucurbita moschata* (Kaddu) and *Capsicum annuum* var. *acuminatum* (Mirch) have been found to contain no crystal of any kind.

Summary

Many of the edible plants contain crystals of calcium oxalate of various kinds. Larger concentrations of the soluble oxalates have been reported to plug the lumen of the tubule causing kidney ailments and producing symptoms of renal insufficiency. Necrosis of epithelial cells occurs when oxalate crystals come in contact with them. Calculi may be formed in the urinary tract. Besides, calcium oxalate crystals may often serve as a character of considerable diagnostic importance. A survey of edible plants have been conducted for noting the presence of calcium oxalate crystals which might aid the dieticians in prescribing desirable vegetables and for those who have a tendency to form stones in digestive or renal systems. The present communication deals with some of the common vegetables indicating presence or absence of crystals, their type and also the distribution in the plant tissues of tne concerned species.

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हिन्दी सारांश

खाद्य वनस्पतियों में कैल्शियम आक्जलेट स्फटिकों का सर्वेक्षण—भाग १

एल० डी० कपूर एवं आर० मित्रा

खाद्य बनस्पतियों में रासायनिक तत्त्वों को निर्माण करने की क्षमता होती है। इनका सेवन हानिकारक हो सकता है। कुछ वनस्पतियों में आक्जेलिक एसिड काफो मात्रा में पाया जाता है। आक्जलेट की अधिक मात्रा मूत्र मार्ग में शर्करा या अश्मरी उत्पन्न कर सकती है। प्रस्तुत लेख में कतिपय खाद्य सब्जियों का अध्ययन किया गया है यथा मूली, शलजम, गाजर, गाँठगोभी, बन्दगोभो, अदरख, प्याज एवं लशुन इत्यादि।

इस अध्ययन में चार प्रकार के स्फटिक पाये गये। परिणाम को चित्रों द्वारा प्रस्तुत किया गया है।

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Jour. Res. Ind. Med. Yoga and Homoeo. 11 : 4, 1976

ON THE BOTANICAL IDENTITY OF SANKHAPUSHPI

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(Received for publication on June 28, 1975).

Introduction

"मण्डूकपर्ण्याः स्वरसः प्रयोज्यः ।
क्षीरेण यष्टीमधुकस्य चूर्णम् ।।
रसो गुडूच्यास्तु समूलपुष्प्याः ।
कल्कः प्रयोज्यः खलु शङ्खपुष्प्याः ।।३०।।
आयुःप्रदान्यामयनाशनानि
बलाग्निवर्णं स्वरवर्धनानि ।
मेध्यानि चैतानि रसायनानि ।
मेध्या विशेषेण च शङ्खपुष्पी ।।३१।।
(इति मेध्यरसायनानि ।)
च० चि० अं० १—३/३०,३१.

The verses quoted above stress the value of Sankhapushpi as a brain tonic, and hold it as superior to mandookaparni and several others.

Sankhapushpi is repeatedly referred to in Ayurvedic writings (Charaka, 1941; Chakradatta, 1955; Vāgbhata, 1956 and Velayudha Kurup, 1972), and occurs as an ingredient in Brahmarasāyana, Aindrarasāyana, Agastyaharitaki, Medhyarasāyana and Mānasamitram (Charaka, 1941, Vāgbhata, 1956 and Velayudha Kurup, 1972)

Inspite of this much popularity, the drug is of disputed botanical identity–'Sandigha'–as is found in the recent literature in Indian Materia Medica (Chopra *et al.*, 1956; Kirtikar and Basu, 1933; Nadkarni, 1954 and Sharma, 1969).

To sum up, the following taxa are regarded as the source of Sankhapushpi :

1. *Convolvulus pluricaulis* Choisy. Convolvulaceae
2. *Evolvulus alsinoides* Linn. Convolvulaceae
3. *Canscora decussata* Schult. Gentianaceae and
4. *Clitoria ternatea* Linn. *Fabaceae*

Though this much of difference of opinion is existing about the exact botanical source of the drug, no attempt has till now been made to ascertain the valid taxon and settle the matter. The present paper approaches this problem, based upon a thorough investigation into the literature and comparative exomorphy of the alleged source plants,

The steps followed in the present elucidation are :

1. Compilation of Ayurvedic synonyms of the drug in Sanskrit, pertaining to the description, use, occurence etc.
2. Enumeration of taxa reported with Sankhapushpi from all available literature.
3. Checking the taxa in item 2 above with the data under item 1 above as to how far the taxa conform to the meaning indicated by the Sanskrit synonyms.
4. Investigation into the current usage of the taxon so arrived at.

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Discussion

The abstracts of description of the four taxa that are reported as Sankhapushpi are as follows (Cooke, 1968) :

1. *Canscora decussata* Schult.

An erect herb upto 50 cms tall with winged stems and ovate or lanceolate, trinerved sessile leaves. Flowers are borne in cymes and are tetramerous with white corolla, Capsule rotund (Figure–1).

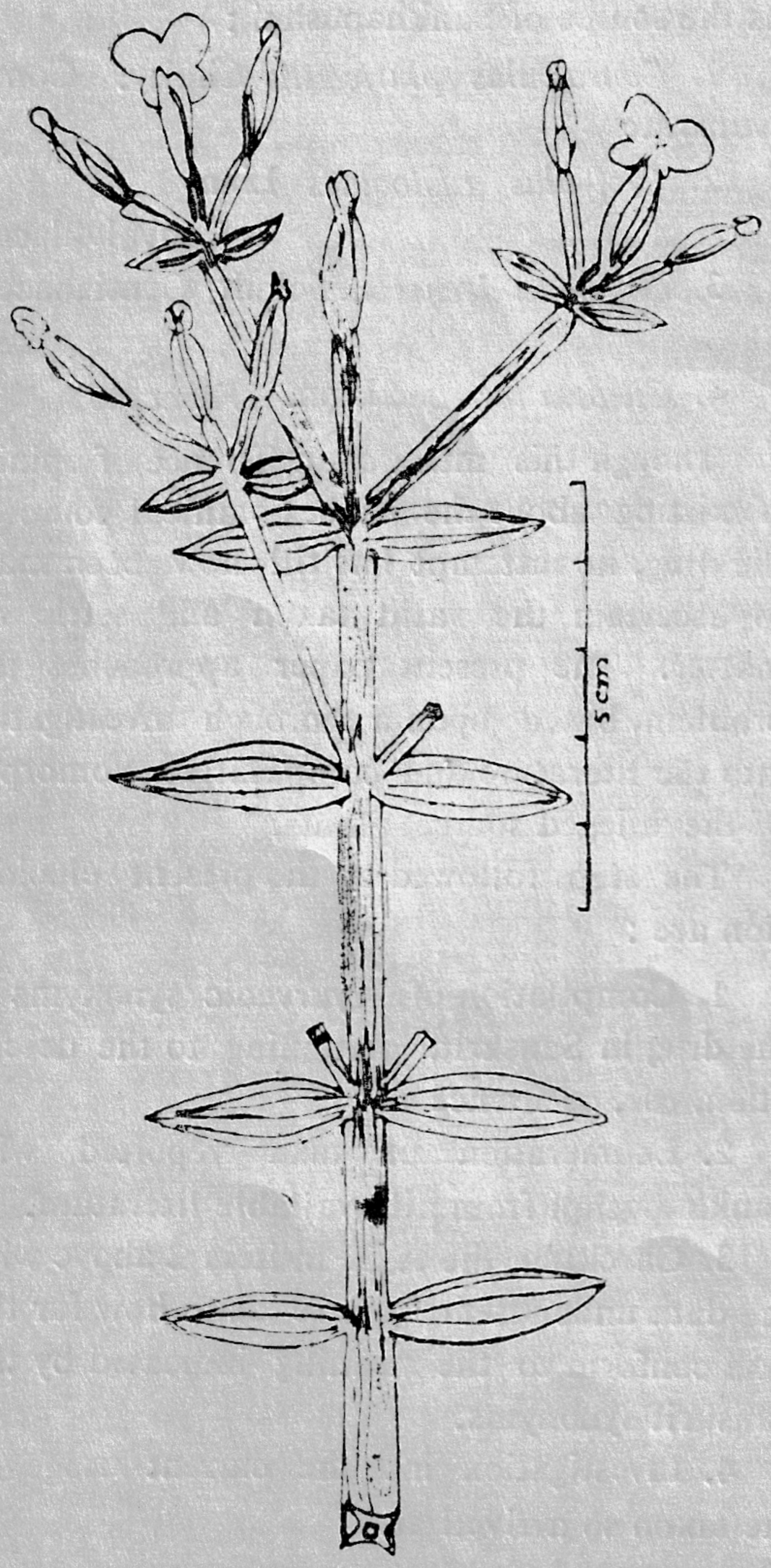

Fig.–1

2. *Convolvulus pluricaulis* Choisy

A procumbent herb with woody base. All aerial parts fulvous. villous. Leaves lanceolate to linear and mucronate. Flowers solitary or a few in upper axils. Corolla broad funnel shaped and white. Capsule globose (Figure–2).

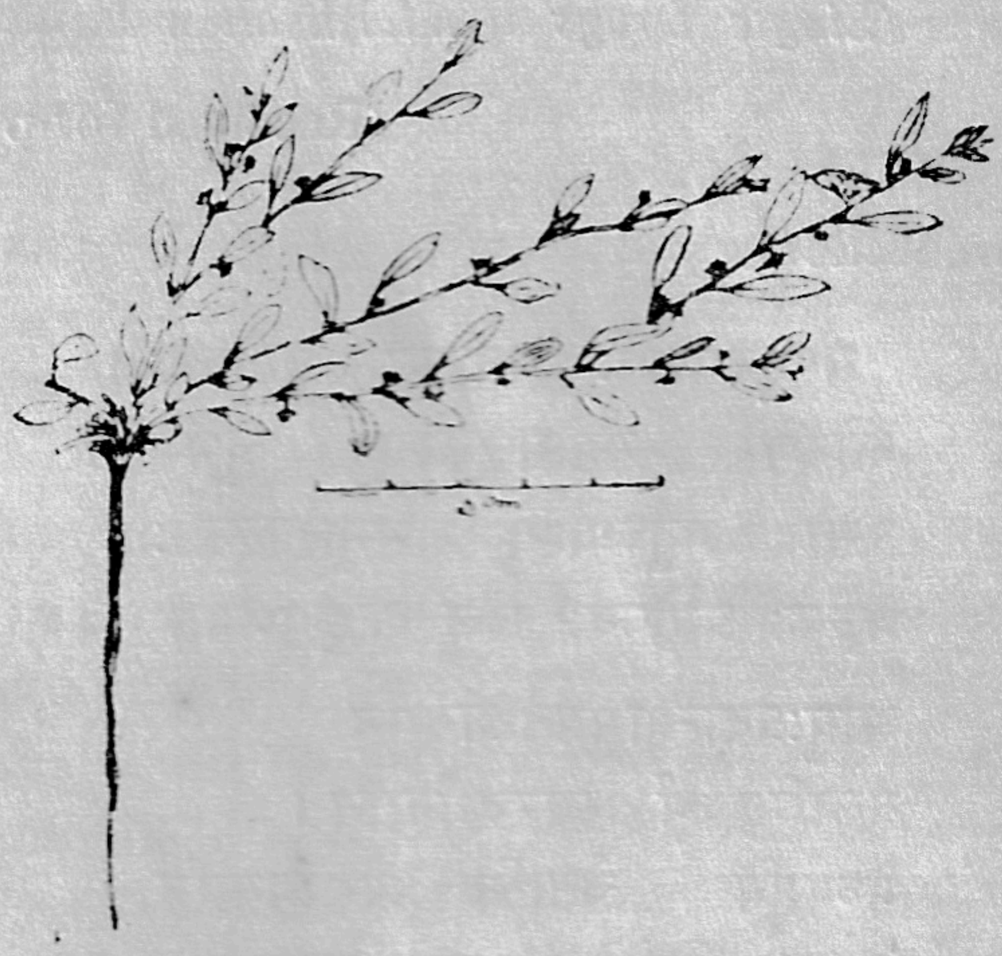

Fig.–2

3. *Evolvulus alsinoides* Linn.

A perennial herb with prostrate branches and small elliptic to oblong lanceolate obtuse mucronate leaves. Flowers mostly solitary in upper axils. Corolla blue, rotate and broad funnel shaped. Capsule globose (Figure–3).

4. *Clitoria ternatea* Linn.

Slender twiner with imparipinnate leaves composed of 5 to 9 leaflets. Flowers axillary solitary and papilionaceous. Standard characteristic with an yellow eye. Flower color cream, white or dark blue or rarely violetish. Pod ensiform (Figure–4).

All the synonyms of the drug 'Sankhapushpi' compiled from the Ayurvedic texts and nighantus were classified according to the meanings indicated by them (Dev, 1961, Williams, 1951), They were tabulated along with the taxa reported to be used or equated

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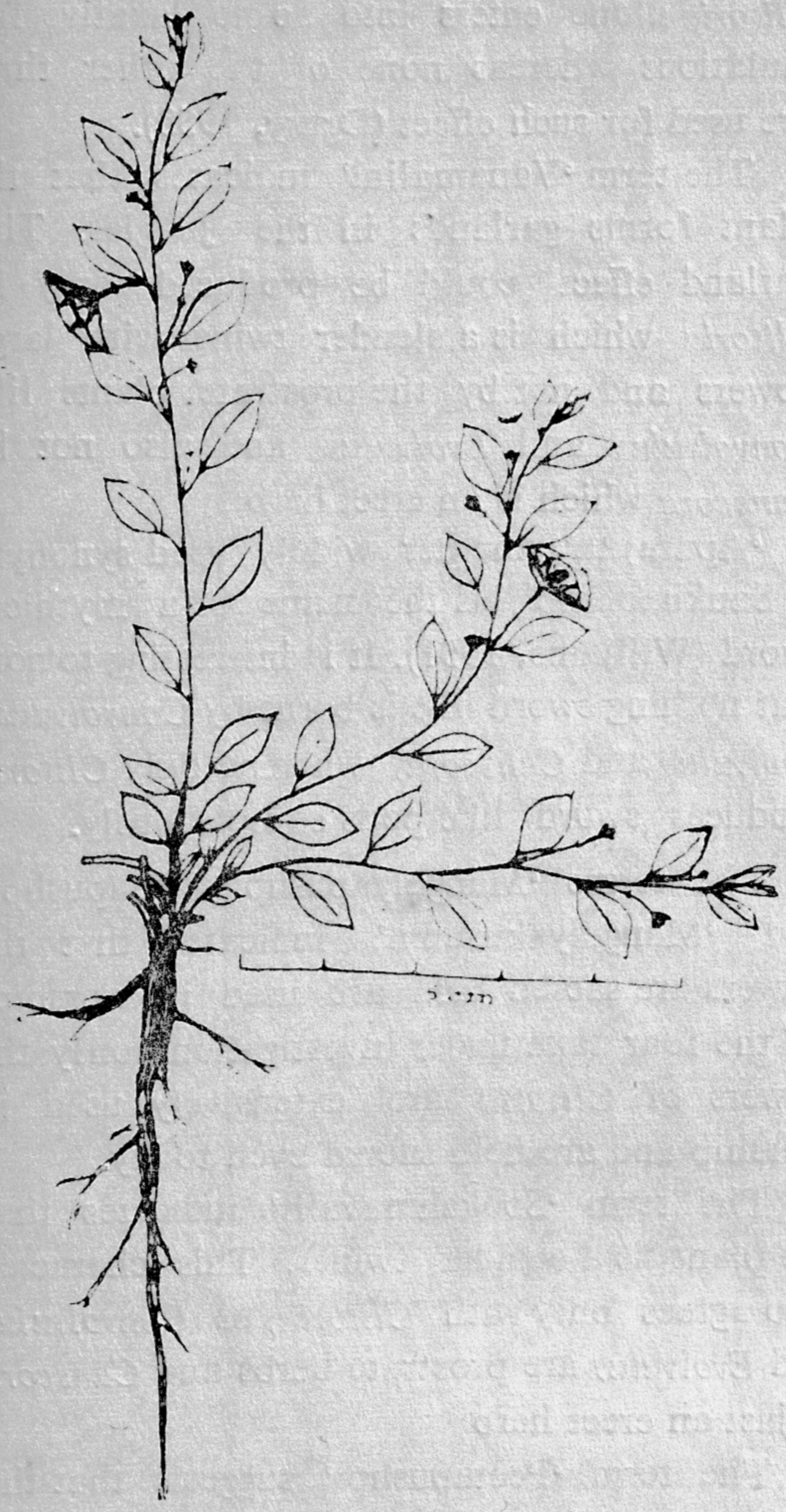

Fig.–3

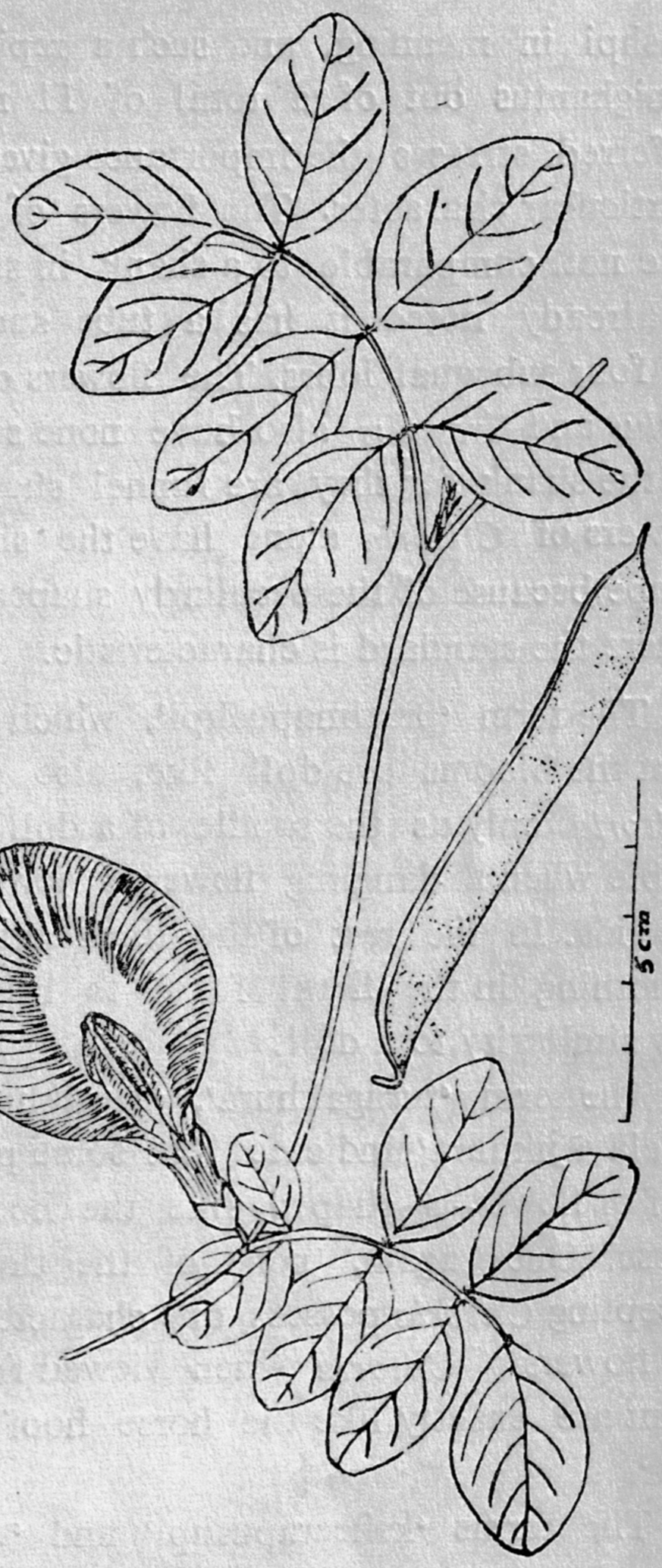

Fig.–4

with 'Sankhapushpi' and checked for the conformity of characters of the plant source with the characters of the drug plant Sankhapushpi as indicated by the various synonyms (Table 1).

From this table it emerges that atleast 29 synonyms with meanings descriptive of the drug plant are available in literature. These synonyms indicate a total of 17 morphological and therapeutic categories.

The synonyms 'Gokarna' and 'Girikarnika' mean that some organ–the leaf or the flower–is like the ear of the cow or the rat in its gross form. When the flower of *Canscora* has a long corolla tube with four subequal lobes and the flowers of *Clitoria* alone has the characteristic standard simulating the pinna (Figure–1).

The term 'Sankhapushpi', which is the most Common in usage to refer to this drug indicates that the flower resembles a conch shell or shank. The terms 'Sankhakusuma', Sankhāhwa, Sankha gālini, kāmbumalini and Kambupushpa are all equivalent to Sankha-

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pushpi in meaning, and such a repitition in 7 nighantus out of a total of 11 nighantus referred, stresses the importance given to this particular character. The flowers of *Conscora* are not comparable to a shank in shape, for, as already noted it has a tube surmounted by four subequal lobes. The flowers of *Convolvulus* and *Evovulus* also have none semblence to the shank for they are funnel shaped. The flowers of *Clitoria* alone have the shank like shape because of the peculiarly shaped corolla where the standard is characteristic.

The term 'pratimapushpi', which denotes that the blooms are doll like, also points to *Clitoria* only as the profile of a doll is discernible when a dangling flower is viewed from the side. In the rest of the three taxa there is nothing in the floral form to bring about any similarity to a doll.

The term 'turagakhura', 'aswakhura' and 'neelavājikhura' indicate that some part—the leaf or flower–is shaped like the hoof of the horse. Once again, none of the three taxa excepting *Clitoria* possess this character. Only the flowers of *Clitoria* when viewed from the front are exactly like the horse hoof in contour.

The terms 'ksheerapushpi' and 'suklapushpi' denote that the flowers are white. For this character, all the four taxa except *Evolvulus* which has blue flowers, answer positively.

However, the other term indicating the flower color and the shape–Neelavājikhura–fits only to *Clitoria* becasse it has blue flowers also with the horse hoof shaped standard combined as shown earlier.

The term 'Malavinasini' indicates that the drug has laxative (sara–Bhavaprakash, 1956)[1] action also. In practice it has been found that *Clitoria* alone enters into some laxative formulations whereas none of the other three are used for such effect (Drury, 1958).

The term 'Vanamalini' indicates that the plant forms garlands in the jungles. This garland effect could be produced only by *Clitoria* which is a slender twiner with large flowers and not by the prostrate plants like *Convolvulus* and *Evolvulus* and also not by *Canscora* which is an erect herb.

'Aparajita', another widely used synonym of Sankhapushpi, is the name of a mythical sword (Williams, 1951). It is interesting to note that nothing sword like is borne by *Convolvulus, Evolvulus* and *Canscora,* whereas only *Clitoria* produces sword like pods conspicuously.

The terms 'Mangalyapushpi', 'Supushpi' and 'Mangalyakusuma' indicate that the flowers are sacred and are used in worship. Of the four taxa under investigation, only the flowers of *Clitoria* are extensively used in worship and are held sacred even today.

The term 'Sookshmavalli' indicates that the plant is a slender twiner. This character also agrees only with *Clitoria,* as *Convolvulus* and *Evolvulus* are prostrate herbs and *Canscora* is just an erect herb.

The term 'Peetapushpi' suggests that the flowers are yellow also in color. The flowers of *Canscora, Convolvulus* and *Evolvulus* have only white or blue flowers but without any yellowness. The flowers of *Clitoria* alone invariably have an yellow patch in the standard, which in the white variety renders the general color of the flowers as cream yellow.

The synonym 'Kireedi' exactly fits to *Clitoria* as the standard petal forms a crown or a hood, when no such structure is existing in the other three taxa.

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1. शङ्खपुष्पो सरा मेध्या वृष्या मानसरोगहृत् । 269.
भा० प्र० मिश्रप्रकरण० गुडूच्यादिवर्ग० ।

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TABLE 1

S. No.	Synonyms and references*		Meanings of synonyms	*Canscora decussata*	*Convolvulus pluricaulis*	*Evolvulus alsinoides*	*Clitoria ternatea*
1.	Gokarna	9	Flowers like ear of	No	No	No	Yes
2.	Girikarnika	9,2,1	Cow or rat				
3.	Sankhapushpi	4,8,7,5,6,3,10					
4.	Sankhakusuma	10	Flowers like a	No	No	No	Yes
5.	Sankhāhwa	4,8,7,3,10	conch shell or shank				
6.	Sankhagālini	10					
7.	Kambumālini	8,7					
8.	Kambupushpi	10					
9.	Pratimapushpi	2	Doll shaped flowers	No	No	No	Yes
10.	Turagakhura	2	Flowers like horse hoof	No	No	No	Yes
11.	Aswakhura	2					
12.	Neelavajikhura	2					
13.	Ksheerapushpi	7	White flowers	Yes	Yes	No	Yes
14.	Sukhlapushpi	5					
15.	Mangalyapushpi	7,6	Sacred flowers	No	No	No	Yes
16.	Supushpi	,10					

(Continued)

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17.	Mangalyakusuma	3,4,10					
18.	Sookshmavalli	11	Slender twiner	No	No	No	Yes
19.	Peetapushpi	10	Yellow flowers	No	No	No	Yes
20.	Kireedi	10	Hooded or crowned flower	No	No	No	Yes
21.	Neelavājikhura	2	Flowers blue and horse hoof shaped	No	No	No	Yes
22.	Bhoo'akhna	10	Spreading on ground	No	Yes	Yes	Yes
23.	Bhoomilakhna	5,9,10					
24.	Malavināsini	10	Laxative / purgative	No	No	No	Yes
25.	Medhya	7,10	Toning the brain or the mental faculty	?	?	?	Yes
26.	Vanamālini	7,10	Garland of the jungles	No	No	No	Yes
27.	Vanavilāsini	7					
28.	Aparājita	1,9	A mythical sword	No	No	No	Yes
29.	Gavākshi	9.	Eye of the cow	No	No	No	Yes
30.	Āsphota	1,9	Arms-clapping of	No	No	No	Yes

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1. Amarakosa, 2. Abhidhanamanjari, 3. Ayurvedachintamani, 4. Bhavaprakasa nighantu, 5. Dhanwantari nighantu, 6. Dravyaguna vignana, 7. Madanapala nighantu, 8. Rajanighantu, 9. Saligrama nighantu, 10. Saligrama nighantu-anyamata, 11. Shivadatta,

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The term 'Āsphota' could refer to the form of mature buds of *Clitoria* which look like clapped arms because of the folded standard.

The term Gavākshi' accurately depicts the flower of *Clitoria ternatea* Linn for that alone resembles the eye of the cow in shape and in coloration. There is nothing in the rest of the taxa to even approximate or suggest the shape of an eye.

The synonym 'Medhya' indicates the therapeutic naturc of the drug. In peninsular India, especially in Kerala, there is no doubt entertained about sankhapushpi being *Clitoria ternatea*. Linn. The Ayurvedists without exception consistantly employ only *Clitoria ternatea* Linn. as Sankhapushpi in their medical practice (Iyer & Kolammal, 1964 and Moose, 1953).

From the foregoing discussion, it becomes clear that only the taxon *Clitoria ternates* Linn. answers positive to all the characters attributed to Sankhapushpi and qualifies itself as the valid source of the drug. *Canscora decussata,* Schultz, *Convolvulus pluricaulis* choisy and *Evolvulus alsinoides* Linn. possess only two or three characters at the most attributed for Sankhapushpi and thus, even this much of coincidence of characters is just chance occurence. In short, these three taxa cannot be accepted as Sankhapushpi.

Summary

The drug Sankhapushpi is a specific brain tonic 'medhya'. But there is difference of opinion about its botanical identity as reflected in the literature and practice of medicine. The literature enumerates *Canscora decussata* Schult., *Convolvulus pluricaulis* Choisy, *Evolvulus alsinoides* Linn. and *Clitoria ternatea* Linn. as the botanical source of the drug.

As any drug has to conform to the maximum with the description, distribution, morphology and organoleptic characters attrributed to the drug in literature, these four botanical taxa were tried as to whether they would tally with such eharacters that are denoted by the synonyms of the drug.

Thus it was found that *Clitnoria ternatea* Linn. alone answers positive to all the characters denoted by the synonyms compiled from references, whereas none of the other three taxa agree with even two or three characters. It has been concluded based on the study of Ayurvedic literature and an examination of the botanical characters that, out of the four claimmants to the status of Sankhapushpi, *Cliioria ternatea* Linn is the valid taxon to be used as Sankhapushpi. The 'Kerala Sampradaya' where *Clitoria ternatea* Linn. is used as Sankhapushpi confirms this

ACKNOWLEDGEMENT

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हिन्दी सारांश

शङ्खपुष्पी की वानस्पतिक पहचान

एन गोपालकृष्ण पिल्लै

मेध्यानि चैतानि रसायनानि ।
मेध्या विशेषेण च शङ्खपुष्पी ॥
इति मेध्यरसायनानि ।

च० चि० अ० १-३/३०, ३१

इस वाक्य से मेध्यौषधों में से 'शङ्खपुष्पी' की श्रेष्ठता ज्ञात होती है। आज भी शंङ्खपुष्पी पौधा भ्रमात्मक है। नीचे लिखे पौधे शङ्खपुष्पी के नाम से आधिकारिक शास्त्रग्रन्थों में और भारत के अलग-अलग प्रदेशों में व्यवहृत हो रहे हैं।

१. कोनवोलवुलस्स प्लूरिकालिस, चोचिसी ।
(Convolvulus Pluricaulis Choisoy)
N. O. कोणवोलवुलेसिये (Convolvulaceae)

२. इवोलवुलस्स अलसिनायडिस, लिन् ।
(Evolvulus alsinoides Linn)
N. O. कोनवोलवुलेसी (Convolvulaceae)

३. कानस्कोरा डिक्कसेट्टा सेल्टलट्ट ।
(Canscora decussata, Schult)
N. O. जेनसियेनेसी (Gentianaceae)

४. किट्टोरिया टेरनेट्टा, लिन ।
(Clitoria ternatea Linn)
N. O. फाबेसी (Fabaceae)

इन पौधों की सही पहचान के लिए अब तक कोई निश्चयात्मक कार्य नहीं हुआ है। मगर नीचे लिखे तरीकों से इन पौधों का सही परख किया गया है।

१. इन पौधों के बारे में आयुर्वेद शास्त्र और वनस्पति शास्त्र में जो कुछ कहे हैं उनको संश्लेषण करके उनके पर्यायों की व्याख्या करके उसके उपयोग और बढ़ाव को देख कर।

२. ऊपर लिखे पर्यायों की व्याख्या करके तुलनात्मक विश्लेषण करके।

३. ये पर्याय सबसे बढ़कर किस पौधे के लिए अनुयोज्य हैं, इसकी चर्चा करके।

४. इस ओषधि के औषध गुण-वीर्य और उपयोगिता और सामयिक प्रसिद्धीकरणों का विश्लेषण करके।

इन संश्लेषण-विश्लेषण पठन के आधार पर चौथे नाम "क्लिट्टोरिया टेरनेट्टा" को ही 'शङ्खपुष्पी' के नाम से आयुर्वेदिक औषध मानना चाहिए।

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Jour. Res. Ind. Mad. Yoga & Homoeo. 11 : 4, 1976.

A PRELIMINARY STUDY ABOUT THE ANTI-INFLAMMATORY ACTIVITY OF *TINOSPORA CORDIFOLIA*

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(Received for publication on April 21, 1976)

Introduction

While complying the composition of various decoctions used in joint disease from Ayurvedic text books (Charak Samhita, Sushruta Samhita, Yogaratnakar, Vangasen Samhita and Gada Nigraha) it was noticed that the plant *Tinospora Cordifolia* was mentioned in almost all decoctions. The present study was undertaken to evaluate the anti-inflammatory activity of the aqueous extract of *Tinospora Cordifolia* by the methods of cotton pellet granuloma (Finney and Somers, 1958) and Formalin induced arthrltis (Buttle *et al.*, 1957). Dexamethasone and indomethacin were used as reference drugs for comparing the anti-inflammatory activity of *Tinospora* Cordifolia.

Materials and Methods

PREPARATION OF THE AQUEOUS EXTRACT

The green stems of the plant *Tinospora Cordifolia* (N. O. Menispermaceae) were collected, crushed and weighed. Water was added to the crushed stems in volumes approximately four times the weight of the stems. This was then allowed to boil on slow fire till the volume of aqueous extract was reduced to about quarter of the original volume. The decoction of the drug was then strained through a thin cloth and the strained fluid was again boiled on slow fire till all the water content was evaporated leaving behind a brownish black residue which was dried and powdered, Fresh suspensions of this powder (12.5 per cent) were made on the day of the experiment by adding 320 mg of gum tragacanth as a suspending agent for each 8 ml of suspensions.

COTTON PELLET GRANULOMA METHOD

Male albino rats (200–250 gm) were kept fasting overnight before the day of experiment allowing free access to water. The rats were lightly anaesthetized with ether (anaesthetic) and under aseptic conditions, four sterilized cotton pellets of 5 mg each, were implanted subcutaneously in groins (one in each) and axillae (one in each). 56 such rats were divided into the following six groups, group one (control group) consisted of 17 rats which were given only gum tragacanth 320 mg/kg by introducing a catheter into the stomach. Group two, three and four consisted of 8, 9 and 8 rats respecti-

1. Head of the Department.
2. Research officer.

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vely and were given graded doses (250 mg/kg, 500 mg/kg and 1 mg/kg) of aqueous extract of Tinospora Cordifolia. Similarly group 5 and 6 consisted of 7 rats each and they were administered indomethacin (1 mg/kg) and dexamethasone (.03 mg/kg) respectively. The first dose of the various agents was administered 6 hours after the implantation of the pellets and subsequent doses were given at an interval of 24 hours for 3 days.

On the day 5 after the implantation of the cotton pellets, the animals were sacrificed by stunning and cutting the blood vessels of the neck. The cotton pellets slong with granulation tissue were carefully dissected out and dried in an oven at 60°C overnight. The pellets were then weighed and difference in the weight of the cotton pellets was interpreted as the weight of the granuloma.

FORMALIN INDUCED ARTHRITIS

Male albino rats (100–150 g) were divided into four groups of eight each. 0.05 ml of 3.5 per cent of Formalin in .9% sodium chloride was injected under the planter aponeurosis of the left hind paw and the same volume of.9% sodium chloride was similarly injected on the right hind paw which served as control (Northover and Subramanian, 1961). The paw volume was determined by immersing it into the fluid in the microburette (Buttle et al., 1957) and measuring the amount of fluid displaced. The inflammatory response due to Formalin was measured as the difference between the volume of fiuid displaced by left and right hind paw. As the development of maximum inflammation due to Formalin occured after 5 hours, the rats in the various groups were given gum tragacanth (320 mg/kg), aqueous extract of Tinospora Cordifolia (1g/kg), dexamethasone (0.03 mg/kg) and indomethecin (1 mg/kg) respectively, after measuring the inflammatory change due to Formalin. The administration of the various agents was repeated every 24 hours for 3 days after the initial dose which was administered after noting the inflammatory change due to Formalin while the inflammatory change was subsequently measured at the end of 29, 46, 53, 77, 94 and 118 hours after the initial injection of Formalin.

Students paired and unpaired 't' test (Bancroft, 1965) was employed to find out the level of significance. The values of p less than .05 were interpreted to be significant.

The drugs employed in the present investigations were dexamethasone (Cadilla), indomethacin (Bombay Tablet Mfg. Co.) and Gum tragacanth (Alembic Chemicals).

Results

COTTON PELLET GRANULOMA METHOD

The results are depicted in Fig. 1. It is evident from Fig. 1 that the aqueous extract of Tinospora Cordifolia caused anti-inflammatory effects when given in doses of 250 mg/kg, 500 mg/kg and 1 g/kg respectively, since the effects produced by these doses differ, significantly ($p<.05$) from those in control rats. However, the effects produced by difierent doses of Tinospora Cordifolia do not differ significantly ($p<1$) from each other. The anti-inflammatory effect caused by 1 g/kg of Tinospora Cordifolia was comparable with that of indomethacin (1 mg/kg) but was significantly ($p<.05$) less than that caused by dexamethasone (0.03 mg/kg).

FORMALIN INDUCED ARTHRITIS

Fig 2 depicts the displacement of fluid due to Formalin induced inflammation as the mean of the difference between the left hind paw and right hind paw. As the value at the

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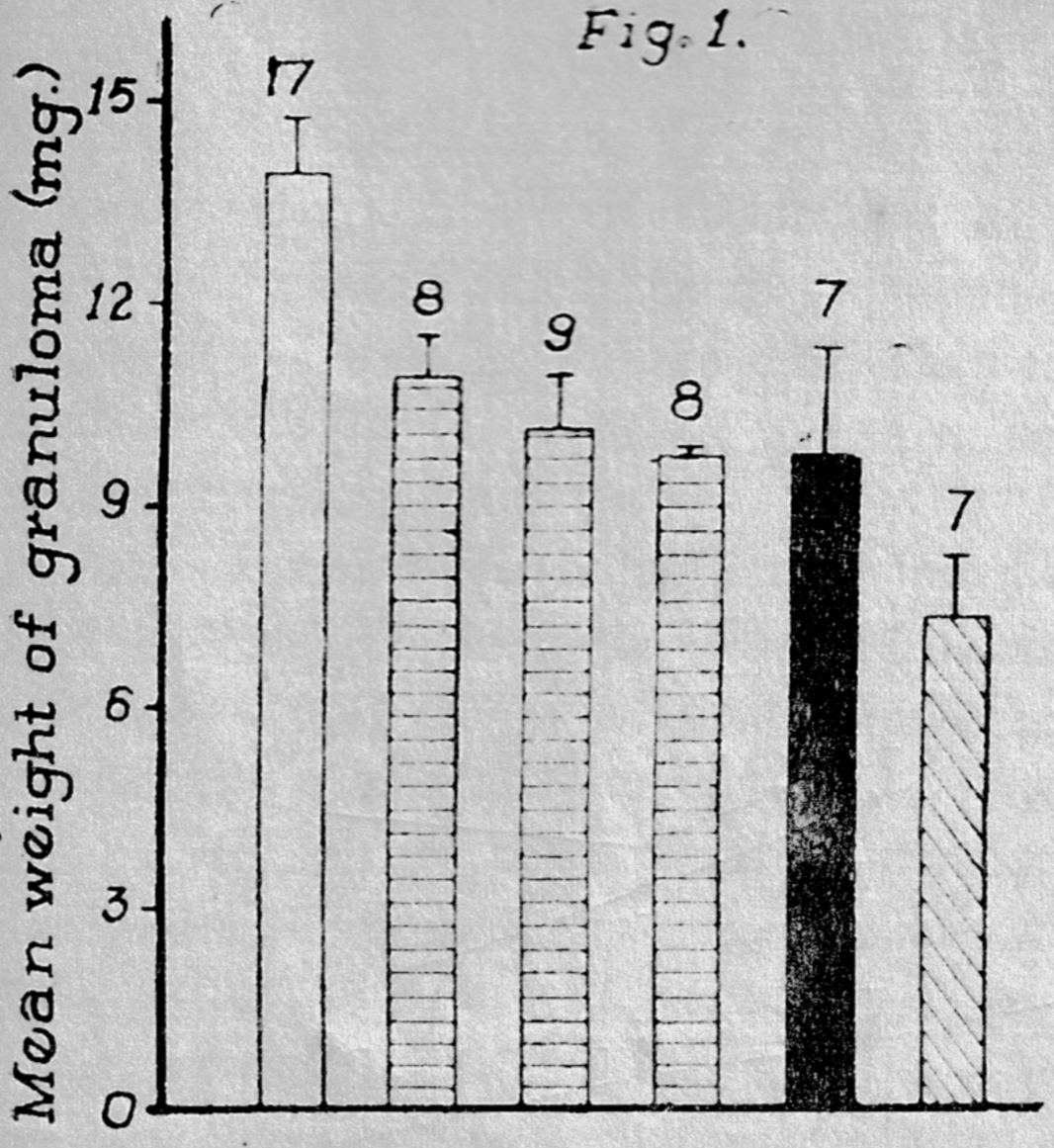
Fig. 1.



Legends

Showing the anti-inflammatory effect of various agents by the method of cotton-pellet granuloma. The mean weight of dried granuloma (mg) is plotted on the vertical axis. The open vertical bar on the horizontol axis depicts the mean weight (mg) of dried granuloma in control rats which were administered 320 mg/kg of gum tragacanth. The three vertical bars with horizontal lines show the mean weight of dried granuloma (mg) obtained due to administration of 250 mg, 500 mg and 1 g/kg of aqueous extract of Tinospora Cordifolia respectively. The solid vertical bar depicts the mean weight (mg) of dried granuloma due to indomethacin (1 mg/kg) while the vertical bar with oblique lines shows the mean weight of dried granuloma in mg due to dexamethasone (.03 mg/kg). The vertical lines and the figures at the top of each bar represent the standard error of the mean and the number of experiments respectively.

end of 5 hours in different groups do not differ significantly ($p<.1$) from one another the other values in the various groups at the end of 29, 46, 53, 77, 94 and 118 hours were compared in the test and the control group by the students paired 't' test. Tinospora Cordifolia (1 g/kg) exerts significant ($p<.05$) anti-inflammatory effect at the end of 29, 46, 77, 94 and 118 hours after the administration of Formalin while the effect at the end of 53 hours is not significantly ($p<.1$) different from that at the end of 53 hours in the control group of animals. Dexamethasone did not cause any significant ($p<.1$) anti-infiammatory effect up to a observation period of 118 hours, while indomethacin showed significant anti-inflammatory effect only before the administration of last (4th) dose at the end 77 hours. The effect of indomethecin was subsequently maintained upto the remaining observation period of 41 hours.

Discussion

The aqueous extract of *Tinospora cordifolia* was evaluated in the present investigation for its anti-inflammatory effects by the method of cotton pellet granuloma and Formalin induced arthritis which represent the proliferative and exudative component of inflammation (Selye and Jasmine, 1956) respectively.

The aqueous extract of *Tinospora Cordifolia* causes a significant anti–inflammatory effect in the cotton pellet granuloma method. meaning thereby that it has an inhibitory effect on the proliferative component of inflammation. The effect caused by 1 g/kg of the aqueous extract is equivalent to that of 1 mg/kg of indomethacein, but significantly less than that of dexamethasone (0.03 mg/kg). The potency difference of about 1000 fold between the aqueous extract and indomethacin is not very

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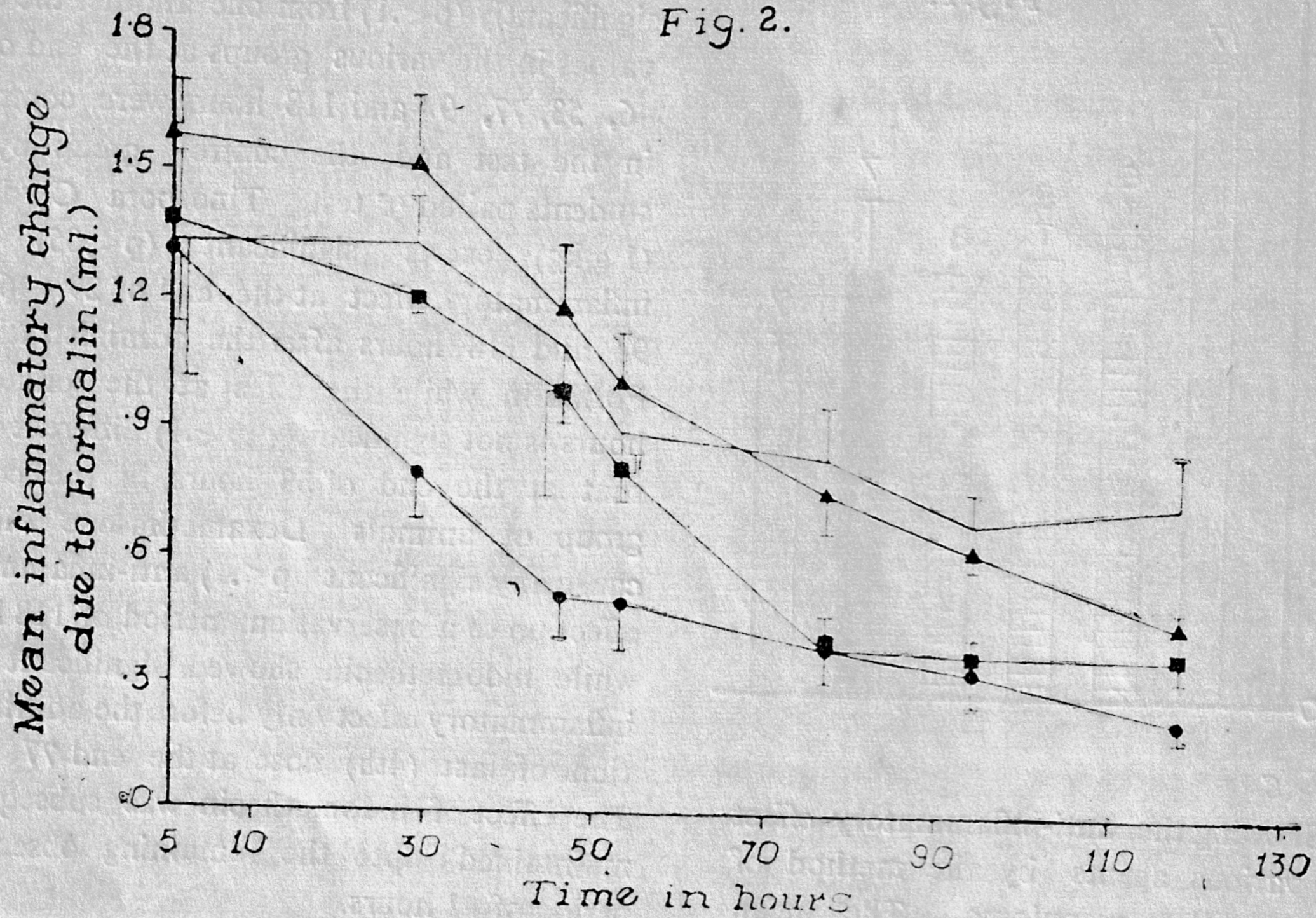
Fig. 2.



Legends

Showing the anti-inflammatory effects of aqueous extract of Tinospora Cordifolia (1 g/kg) indomethacin (1 mg/kg) and dexamothasone (.03 mg/kg) administered orally at the end of 5, 29, 53 and 77 hours against Formalin induced inflammatory change in the left hind paw of rats. The vertical axis depicts the mean inflammatory change due to Formalin (ml) while the horizontal axis show the time in hours from the time of initial injection of Formalin. The solid line depicts the mean inflammatory change due to Formalin in control rats which were administered gum tragacanth (325 mg/kg). The line joining the solid circles shows the mean inflammatory change due to Formalin in rat which were admininistered 1 g/kg of aqueous extract of Tinospora Cordifolia. The line joining solid squares represents the mean inflammatory chnage due to Formalin in rats administered indomethacin (1 mg/kg) while the line joining the solid triangles shows the mean inflammatory change due to Formalin in rats administered dexamethasone (.03 mg/kg). The vertical lines on each point represent the standard error of the mean, while each vertical line represents the mean results of eight experiments.

big taking into account the crude nature of the extract.

As 1 g/kg of aqueous extract was found to cause maximum anti-inflammatory effect in cotton pellet granuloma method, the effect of the same dose was evaluated in the method of Formalin induced arthritis. The anti-inflammatory effect of the aqueous extract was visible within 24 hours of administration of the lst dose and subsequently a significant effect was maintained for the entire period of observation (118 hours) except for the period

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just before the administration of 3rd dose of aqueous extract. Presumably either the effect of the 2nd dose given at the end of 29 hours declines after 17 hours of its administration or it is this period in which the anti-inflammatory response to Formalin showed more than expected decline in the control animals, under present investigation. Gulati *et al,,* (1973), reported a percentage reduction of inflammation in Formalin induced arthritis in control animals as only 3.07 percent at the end of 53 hours while it was 27.16 percent at the end of 118 hours. The corresponding percentage decline in the present investigation was 25.58 percent and 35.13 percent respectively.

In contrast to the effect of aqueous extract of *Tinospora cordifolia,* indomethacin (1 mg/kg) exerted significant anti-inflammatory effect only before the administration of 4th dose at the end of 77 hours. The anti-inflammatory effect of indomethacin was subsequently maintained till the end of the observation period. Dexamethasone (0.03 mg/kg) did not show any significant anti-inflammatory effect against Formalin induced arthritis, throughout the period of observation in the present investingation. This result is in agreement with the observation that salicylates protect against formaldehyde induced inflammation, against which cortisone was ineffective (Davison and Mandle, 1971).

It is concluded therefore, that aqueous extract of *Tinospora cordifolia* exerts anti-inflammatory effects against both the proliferative and exudative component of inflammation. This conclusion is in agreement with observations (unpublished) that when it was administered (1380 mg/day) to the patients having different types of arthritis such as rheumatoid arthritis, osteoarthritis and senile arthritis, about 50 percent of the patients showed relief of symptoms such as improved mobility of joints reduction cf oedema and relief of pain. The aqueous extract seems to be resembling non-steroidal anti-inflammatory agents such as indomethacin rather than corticosteroids.

Summary

The aqueous extract of *Tinospora cordifolia* was evaluated for its anti-inflamatory effect by the methods of cotton pellet granuloma and Formalin induced arthitis, using indomethacin and dexamethasone as reference drugs. The aqueous extract of Tinospora Cordifolia and indomethacin exerted a significant anti-inflammatory effect by both the methods while dexamethasone showed powerful anti-inflammatory effect only in the method of cotton pellet granuloma. The mechanism of action of the aqueous extract of *Tinospora cordifolia* appears to resemble that of a non-steroidal anti-inflammatory agent.

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हिन्दी सारांश

अमृताघन की शोथघ्नता का अध्ययन

डी० एस० साह एवं डी० सी० पाण्डे

अमृताधन की शोथघ्नता का परीक्षण दो प्रकार से किया गया (१) कोटन पेलेट ग्रेन्युलोमा मेथड और (२) फोरमेलाइन इन्ड्यूस्ड-आर्थराइटीस। अमृताधन की शोथघ्नता के तुलनात्मक अध्ययन के लिए इन्डोमेथासीन एवं डेक्सामीथासोन का उपयोग किया गया। गुडीचीधन एवं इन्डोमेथासीन उपयुक्त बतायी हुई शोथघ्नता नामक दोनों प्रयोगों में सफल रहे जब कि डेक्सामीथासोन सिर्फ प्रथम शोथघ्नताशामक प्रयोग में अमोध एवं सफल औषध रहा है। अतः इन परीक्षणों से यह ज्ञात हुआ है कि अमृताधन की शोथघ्नता नोन स्टीरोइडल औषध के समान हो सकती है।

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STUDIES ON THE ANTIFERTILITY ACTIVITY OF *EMBELIN*

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Introduction

In 16th Century Bhava Prakash claimed that Embelia Ribes (Vidanga) possessed oral contraceptive action. Arora *et al.* (1971) reported the significant anti–implantation effect of aqueous extract of Embelia ribes. An active principle Embelin (Embelic acid : 2, 5–dihydroxy–3–undicyl–1, 4–benzoquinone) isolated from the berries of Embelia ribes was found to have anti–fertility effect (Santhakumari and Rathinam, 1975). Radhakrishna Pillai *et al.* (1975) had reported 83.3% anti–fertility effect in rats with embelin 120 mg/Kg body weight given orally during D_1–D_5 of pregnancy. We report here the anti–fertility effects of Embelin on rats and rabbits using much smaller dose of 10 mg/Kg and also its effect on the target organ–viz. Ovary.

Materials and Methods

Isolation of Embelin—Isolation of Embelin was carried out by the C. D. R. S. Chemical Unit, Trivandrum, using the following method :

The powdered seeds (500G) of Embelia Ribes were extracted with petroleum ether (60–80°) in a soxhlet for about 8 hours. The extract (1.5 litres) was filtered and crystallised twice from ethanol to get orange red needles; melting point 142–144°, yield 14.5G. This was identified as Embelin (Embelic acid).

1. ANTI–IMPLANTATION EFFECT

Adult female rats of proven fertility (weight 150–200G) from the animal colony of C. D. R. S. Pharmacological Unit, Medical College, Trivandrum, were used throughout our study. Antifertility screening was done by the method of Khanna and Chaudhury (1968) with slight modification with respect to the days of drug administration. Here we have treated the rats post coitum from D_1–D_5. To the control group 0.5% gum acacia 10 ml/Kg body weight was given orally through an intra gastric tube and to the treated group 10 mg/Kg body weight of Embelin in 0.5% gum acacia as above. On 10th day of pregnancy, laparatomy was performed and the number of implantation sites were noted both in control and treated group. Presence of even a single implantation in a rat on D_{10} was considered as pregnant. The abdominal cavity was sutured and the rats were allowed to deliver full term.

2. ANTI–OVULATORY STUDY IN RABBITS

The anti–ovulatory study in rabbits was carried out in 12 adult (1.2–1.5 Kg) non–pregnant female rabbits kept separately for three weeks before use. Copper acetate (4 mg/Kg) was given intravenously in their marginal ear vein to induce ovulation (Khanna and Chaudhury, 1968). 10 mg/Kg of Embelin in

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0.5% gum acacia was given orally for 5 days to the treated group of 6 rabbits. Control group consisting of 6 rabbits received only 1 ml/Kg of 0.5% gum acacia orally for 5 days. On 6th day Copper acetate (4mg/Kg) was given intravenously to induce ovulation in all rabbits. After 48 hours of Copper acetate administration laparotomy was done in all Rabbits and their ovaries were examined for number of bleeding points both in control and treated groups.

3. OESTROGENIC EFFECT

Oestrogenic effect of Embelin (10 mg/Kg) has been performed as per the method of Bulbring and Burn (1935). 16 female rats (80–100G) were ovariectomised and kept for 10 days and daily vaginal smear was examined. When all the rats showed continued dioestrus stage of oestrus cycle, to one group 0.5% gum acacia 10 ml/Kg body weight and to the other Embelin 10 mg/Kg in 0.5% gum acacia and to the third group 0.5% gum acacia 10 ml/Kg body weight orally and 5mcg. of Oestradiol benzoate I/M was given for 5 days. On D_6 all rats were killed and uteri of different groups were kept in Bouin's solution and on the next day they were dissected out and dried using filter paper and weighed accurately. Oestrogenic effect was assessed by the utrotropic activity of the test compound as compared with the control and standard and also by observing the changes of the vaginal smear in both groups. The weight of each uterus was calculated per 100G body weight of rat.

4. ANTI-OESTROGENIC EFFECT

Two groups each of 6 adult ovariectomised rats weighing 80–100G were taken and treated for 5 days as follows :

Group I—Control—Oestradiol Benzoate 5 mcg. I. M. +0.5% gum acacia 1 ml/100G body weight orally.

Group II—Treated—Oestradiol Benzoate 5mcg. I. M. and Embelin 1 mg/100G body weight orally.

Vaginal smears were taken during the period of treatment. The animals were weighed and autopsied on 6th day. Uteri were removed and preserved in Bouin's solution for 24 hours, dried between filter papers and weighed accurately. Anti–oestrogenic activity was assessed by the decreased utero–trophic activity in Embelin treated group. Results were analysed statistically using the student's 't' test.

5. ACUTE TOXICITY STUDY

Embelin in doses of 10 mg–3G/Kg. was given orally to rats and mice and effects studied for 72 hours.

6. SUB–ACUTE TOXICITY STUDY

10 weeks administration of 10mg/Kg of Embelin to rats and studied the Hb%, WBC, RBC count and Histology of heart, liver, kidney, spleen and adrenal.

Results

1. ANTI–IMPLANTATION EFFECT

Embelin in doses of 10 mg/Kg given from D_1–D_5 of pregnancy completely prevented implantation in all rats (Table 1).

2. ANTI–OVULATORY EFFECT

1 mg/Kg of Embelin showed highly significant reduction of bleeding points ($P<0.001$) in the ovaries (Table 2) as compared to control animals.

3. OESTROGENIC EFFECT

Embelin treated group of rats showed no significant ($P>0.1$) oestrogenic effect when compared to the control group which received 0.5% gum acacia 10 ml/Kg while those receiving 5 mcg. of oestradiol monobenzoate showed highly significant utero tropic activity ($P<0.001$). (Table 3). Vaginal smear study also corroborated this finding (Table 3 a).

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TABLE 1

Anti-Implantation effeci of embelin 10 mg/kg given orally from day 1–day 5 of pregnancy in female albino rats

Drug	Dose mg/kg body weight	No. of rats used	No. of rats showing implantation on 10th day of pregnancy.	No. of implantation sites in individual rats on 10th day of pregnancy.	% of anti-implantation activity
1. Control (0.5% gum acacia)	10ml	6	6	7,6,8,7,9,6.	0
2. Embelin	10 mg	6	0	——	100

TABLE 2

Anti-Ovulatory effects of embelin 10 mg/kg in coppor acetate induced ovulation in rabbit

Sl. No.	Drug/kg vehicle	No. of rabbits taken	No. of bleeding points in the ovaries of each rabbits	Mean No. of bleeding points ± SE.	t	P
1. Control	0.5% Gum acacia 10 ml.	6	7,8,5,6,4,6	6± 0.58		
					6.17	<0.001
2. Treated	Embelin 10 mg	6	2,1,3,0,2,0	1.3± 0.50		

TABLE 3

Oestrogenic Effect of Embelin 10 mg/kg Body Weight Orally in Rats Against 5 mcg. of Oestradiol Treated Group

Sl. No.	Drug/Kg	No. of Rats used	Weight of the uterus in mgs. in 100 gm body weight	Mean vatue ±S. E.	t	p
1.	Control 0.5% gum-acacia 10 ml.	5	85, 110, 130, 120, 130	115±8.35	1.548	>0.1 (Not significant)
2.	Embelin 10 mg + 0.5% Gum acacia 10 ml.	5	110. 100, 140, 100, 200	110±7.7		
3.	0.5% gum acacia 10 ml + 5 mcg of Oestradioi	6	175, 173, 170, 165, 75, 165, 75, 176, 50.	172.7 ±2.4	6.6	<0.001 (Significant)

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TABLE 3 a

OESTROGENIC ACTIVITY.

Effect of Embelin 1 mg/100 gm Body Weight Orally on the Vaginal Smears of Overiectomiscd Albino Rats as Compared to the Rats Receiving Vehicle (0.5% Gum Acacia) 1ml100 gm Body Weight Orally.

No. of Rats	Control (0.5% Gum acacia 1ml/100 gm bodyweight) Days of Examination.										Embelin Treated (1mg/100Gm body weight) Days of Examination.									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
1	M	P	D	D	D	D	D	D	D	D	P	P	D	D	D	D	D	D	D	D
2	P	D	D	D	D	D	D	D	C	D	P	D	D	D	D	D	D	D	D	D
3	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D
4	P	P	P	D	D	D	D	D	D	D	M	P	P	D	D	D	D	D	D	D
5	M	M	D	D	D	D	D	D	D	D	P	P	D	D	D	D	D	D	D	D
6	M	P	P	D	D	D	D	D	D	D	M	M	D	D	D	D	D	D	D	D

D—Diestrus stage of Estrus cycle.
M—Meta–estrus stage of Estrus cycle.
E—Estrus stage of Estrus cycle.
P—Pro–estrus stage Estrus cycle.

4. ANTI–OESTROGENIC EFFECT

Embelin did not produce any significant anti–oestrogenic effect (Table 4). Vaginal smear examination also did not produce any change due to Embelin (Table 4 a).

TABLE 4

Anti-Oestrogenic Effect of Embelin on Rats

Sl. No.	Particulars	Drug/Kg vehicle	No. of rats used.	Weight of uterus in mgs/100 gm body weight.	Mean value S. E.	t	p
1.	Control	0.5%Gum acacia + 5 mcg Oestradiol monobenzoate I. M.	6	175, 173, 170, 165, 75 165.75 176.50	172.7± 2.45	1.5	∠0.2
2.	Treated	Embelin 10 mg + 5 mcg. Oestradiot monobenzoate I. M.	6	180,174,5, 190,5 175.75,164.25,195	180 ± 4.64	Not significant.	

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TABLE 4 a

ANTI-OESTROGENIC ACTIVITY

Effect of embelin (1 mg/100gm body weight orally) and oestradiol (5 mcg/100gm body weight I.M.) on the vaginal smears of ovariectomised rats as compared to oestradiol (5 mcg/100gm body weight I.M.) alone.

No. of rats.	Oestradiol – Control Days of Examination.					Embelin + Oestradiol Treated Days of Examination.				
	1	2	3	4	5	1	2	3	4	5
1	P	E	E	E	E	M	D	P	E	E
2	M	D	P	E	E	D	P	E	E	E
3	D	D	P	E	E	P	E	E	E	E
4	M	D	P	E	E	M	D	E	E	E
5	D	P	E	E	E	P	E	E	E	E
6	P	E	E	E	E	D	P	E	E	E

5. ACUTE TOXICITY STUDY

In rats and mice Embelin with dose upto 3G/Kg body weight orally did not show LD_{50} effect, which indicates its possible wide margin of safety.

6. SUB–ACUTE TOXICITY STUDY

10 weeks administration of Embelin 10 mg/Kg orally to rats showed no significant change in histology of heart, liver, kidney, adrenal and spleen, except non–specific hyper–plasia of splenic cells with Haemocydarin frag–mentation which are the characteristrics of Haemorrhage. In blood picture also no significant change was observed in the RBC and WBC counts and Haemoglobin levels.

Discussion

It appears from the above results that Embelin (10 mg/Kg) given orally has significant (100%) anti–implantation effect in adult female rats. It is also exhibiting highly significant ($P<0.001$) anti–ovulatory effects in rabbits. Embelin has no significant ($P>0.1$) Oestrogenic effect or anti–oestrogenic effect which is contrary to the findings of Arora *et al.* (1971) who observed such an effect with Embelia Ribes aqueous extract. The drug seems to have no effect on the ovary. It may be preventing the release of oestrogen and progesterone during early phase of pregnancy where these hormones are essential for implantation of fertilised eggs Mayer, 1963. It has been observed by Chakravarthy (1961) that Oracin (containing by weight of Embelia ribes 50%, piper longum 30%, Borax 15% and asofoetida 5%) markedly inhibited the effect of oestrogen in mobilising the alkaline phosphatase activity in the endo–metrium. Whether such an inhibition of alkaline phosphatase activity is the above action in preventing conception is not yet proved. Further studies are in progress to elucidate its mechanism of action.

Summary

Antifertility studies on Embelin showed that in 10 mg/Kg. dose given orally to albino rats from D_1–D_5 of pregnancy it had 100% anti–implantation activity. The same dose

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of Embelin given to rabbits also exhibited highly significant ($P<0.001$) anti–ovulatory effect, but no significant ($P>0.1$) oestrogenic or anti–oestrogenic effects in rats. Embelin was found to have a high margin of safety in acute toxicity studies in rats. Even at a high dose of 3 g/Kg. orally it did not show any toxic effects. Sub acute toxicity also indicated the drug to be free from toxic side effecs on bone–marrow, liver, kidney and heart.

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हिन्दी सारांश

एम्बेलीन के प्रजननशक्ति विरोधक कार्य का अध्ययन

के० रथिनम्, जी० शान्ता कुमारी एवं एन० रमैया

भाव प्रकाश में विडंग का प्रयोग गर्भनिरोधक औषधि के रूप में किया गया है। अरोड़ा इत्यादि (१९७१) ने भी इसका अध्ययन किया। बाद में अन्य वैज्ञानिकों ने भी इसका अध्ययन किया। प्रस्तुत अध्ययन में एम्बेलीन नामक तत्त्व का चूहों एवं शशकों पर प्रजननविरोधी कार्य के लिए प्रयोग किया गया। इसकी मात्रा १० मि० ग्रा०/कि० ग्राम दी गयी। पर्याप्त महत्त्वपूर्ण परिणाम पाये गये।

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Jour. Res. Ind. Med. Yoga & Homoeo. 11 : 4, 1976.

Short Research Communication

CATHARANTHUS ROSEUS–A USEFUL MEDICINAL PLANT

Introduction

Catharanthus roseus belongs to family Apocynaceae. Medicinal Plants of this family namely Rauwolfia serpentina, stropenthus his, Thevesia nerii, Oleander nerium Holarrhena anti-dysenterica, Alstonia scholaris, Apocynum can. etc. are possessing ample therapeutic potentialities.

Catharanthus roseus (vinca rosea linn.) has enjoyed a wide reputation in folk medicine and rapidly gaining importance in the modern therapeutic world for its effective clinical uses in certain dangerous diseases & complaints.

Observations

The Plant has enjoyed a popular reputation in indigenous medicine in various parts of the world. 'The fresh leaves are poisonous to grazing cattles as well as cardiac depressant' [23]*C. roseus* also occupies a fairly top position in the Indian exports list of raw materials of therapeutic value and has established a definite position in the indigenous medicine throughout the world for its antitumour, antimalignant, antileukaemic; diuretic & hypotonic properties.

VERNACULAR NAMES

The followings are the common vernacular names of the species. Hindi-sadabahar, Bengali-Nayantara, Punjabi-Rattangot, Oriya, Ainskati, English-Periwinkle, Marathi-Sadaphul, Telegu-Billaganneru,

DESCRIPTION

"*C. roseus* is a fast growing shrub, woody at the base, and 40-80 cm. high with erect branches. Leaves are opposite, oblong and petiolate, deciduous having acute base and rounded or mucronate tip. The plant is pubescent when young and glabrous when mature. Flowers are violet, rose or white. Fruit, is mostly 10-20 seeded follicles when dried, the leaves become yellowish green & brittle. The powder of the leaves of *C. roseus* is deep green and without any appreciable taste. Odour is some-what Pungent" *Parts used*-whole plant.

DISTRIBUTION

The plant is probably native of tropical America but now naturalized in many parts of the world. It is also widely cultivated in the gardens throughout the globe as an ornamental horticultural plant. 'The plant *C. roseus* is not indigenous to our country but still it is often found through out India either as a cultivated garden plant or growing wild in sandy soil.' The plant is common in western Hlmalayas, upper Gangetic plain, Bihar, Orissa, Sind, Gujarat, Konkan, Deccan and Karnatak. 'Cultivation in garden at Bharwain'

MEDICAL USES, ACTIVE CONSTITUENTS AND THEIR BIOLOGICAL ACTIONS

The plant has enjoyed a popular reputation in Indigenous medicine in various parts of the world. 'An early description of the medicinal

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properties of this plant mentions that the juice of the leaves was employed as an application to wasp or bee stings. An infusion of the leaves was used in the control of Haemorrhage and scurvy, as a mouth wash for toothache & for healing and cleaning chronic wounds and dermatitis'. The plant part (root, leaf.) or whole V. Roseus is being used by the local people in the rural area of different part of India & aboard for getting relief from ailments like—Diabetes, Dysentery, Dyspepsia, Menorrhagia, Skin infection, etc. and in folk medicine it is used as abortive agent, astringent, antigalactogogue, in fever, toothache, urinary antiseptic, vermifuge.

'The leaves are used externally for subsiding the swelling of the different parts of the body'.

"The most striking clinical results of VL B (Vinca-leukoblastine) therapy have been obtained in the treatment of Hodgkin's disease, Choriocarcinoma and carcinoma of the breast have also been effectively treated." (Llodyia 1965).

"Two new dimetric alkaloids leurisidine and Leurocristine have been obtained. Both compounds are extremely active against P. 1534 Leukaemia in DBA/2 mice, giving indefinit survivors" (Lloydia. 1961).

'Some of the alkaloids are very effective and on animal or human application have induced a dramatic remission in a wide range of tumours and malignant growths including carcinoma of the Lung, Kidney, breast, ovary and cervix, Chorioepithelioma, rhabdomyosarcoma, reticulum cell sarcoma and neuroblastoma. Carcinomas of the stomach, colon and rectum, melanoma, teratoma, monocytic leukemia, erythroleukemia, astrocytoma and mycosis fungoides have also responded to its application'.

'During recent years the plants C. roseus has come into prominence in the medical world as the Plant yields an alkaoid vincaleukoblastine ($C_{46}H_{58}N_4O_9$ – which is used for the treatment of Hodgkin's disease & choriocarcinoma'.

It has also been reported that the total alkaloids possess a limited antibiotic activity against vibrio cholerae and M. Pyogens Var. aureousas an antiviral agent as well as a significant and sustained hypotensive action.

The leaf extract was reported to cure diabetic ulcers and as an effective oral hypoglycemic (blood sugar reducing) agent.

The macerated root was given as atonic and stomachic. It was also used in treating menorrhagia. 'Preliminary screening of perivincine for hypotensive activity in anaesthetic rats indicates that the alkaloids produces a rapid transient drop in carotid blood pressure, they have also depressant action on the central Nervous System.

"Regarding the antidiabetic activity of the drug. the anti-diuretic effects of the alkaloidal fractions may be mistaken for an action against diabetes".

THE CHEMISTRY-VINCA ALKALOIDS

Vincaleukoblastine (Noble et al 1958)
Vinblastine (Vincaleukoblastine, VL B.)
Vincristine (V. CR), Vinleurosine (V.L.R)
And Vinrosidine (V R D).

Due to the advance in chromatography, thin iayer chromatography, electrophoresis and sophistication in instrumentation, Over 65 different alkaloids of a new class of oncolytic agents have been isolated from this plant.[23]

'Percentage of alkaloids[28]'.	In roots	In leaves	In stem
Pink in variety	1.08	0.82	0.32
White variety	1 34	0.74	0.17

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VINKA ALKALOIDS AND THEIR BIOLOGICAL ACTIVITY (13.23)

Biological activity—

(A) Anti Leukaemic

1. Leurocristine—($C_{46}H_{54}N_4O_{10}$. 2) Leurosine – $C_{46}H_{58}N_4O_9$.
2. Vincaleucoblastine—$C_{46}H_{58}N_4O_9$ (anti leukemic, Hodgkins disease, Choriocarcinoma.
3. Vinblastine—$C_{46}H_{56}N_4O_9$ (antileukaemic, anticancerous).
4. (i) Vincristine—$C_{46}H_{54}N_4O_{10}$ (Oncovin Drug U. S. A).
   (ii) Leurocristine—do.
5. Vindolicine – $C_{25}H_{32}N_2O_6$.
6. Vindolidine—$C_{48}H_{64}N_2O_{10}$.

(B) Anti Cancerous

1. Vinblastine—$C_{46}H_{56}N_4O_9$ (anti leukaemic and anti cancerous).
2. Vindoline—$C_{25}H_{32}N_2O_6$.
3. Vindolinine—$C_{21}H_{24}$ – 6 N_2O_2.

(C) Antitumorous

1. Carosidine—Dimeric.
2. Carosine—$C_{46}H_{56}N_4O_{10}$.
3. Catharanthine—$C_{21}H_{21}N_2O_2$.
4. Lochneridine—$C_{20}H_{24}N_2O_3$.
5. Vinleurosine—$C_{46}H_{58}N_4O_9$.
6. Virosine—$C_{22}H_{26}N_2O_4$.

and other alkaloids possessing antibacterial, Hypoglycemic, Hypotensive, antidiuretic sedative–biological activity are not mentioned here.

These are quoted from (23,13) Journal of the Science Club Vol. XXV Nos. 1 and 2, 1971 P. P. 31–36 and Jonson *et al.* 1963. The Minor differences in structure of few alkaloidal molecules result in notable differences in toxicity and antitumor spectra.

Removal of the acetyl. Group of end of one portion of vinblastine destroys its antileukemic activity' 31.

CLINICAL TOXICITY

'The clinical toxicity of vincristine is mostly neurological with paresthesia, loss of deep-tendon reflexes, neurotic pain, muscle weakness that may be menifested by foot drop and inability to walk, hoarseness, ptosis, and double vision'.

'Occurrence of the earliest symptoms–usually tingling and numbness of the extremities, severe constipation, sometimes resulting in colicky abdominal pain'

'Alopecia occurs in about 20% of patient given Vincristine, however, it is always reversible, frequently even without cesation of therapy'.

'Although not nearly as common as with vinblastine, leukopenia may occur with vincristine, and thrombocytopenia and anaemia has been reported occasionally'

Different Species of the Genera vinca are known as—

1. Vinca Rosens-Linn. (Catharanthus rosens).
2. Vinca Minor. (Lesser Periwinkle).
3. Vinca Pusilla–Murr. (Catharan thus Pusilla).

HOMOEOPATHIC SYMPTOMATIC CLINICAL INDICATIONS OF VINCA MINOR

Skin–This remedy is noted for skin affections of various kinds but the most important field of its action is located on thc scalp. It has been used with great success in crusta lactea, favus, plica polonica, eczema and alopecia and spots on scalp, oozing moisture matting hair together corrosive itching of scalp. Great sensitiveness of skin with redness and soreness from slight rubbing.

Nose—'The slightest excitement or mental emotion throws a quantity of blood into the nose and it looks almost purple'.

Throat—'Difficult swallowing ulcers, Diptheria'.

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Female—Excessive menstruation with great weakness. Passive uterine haemorrhages. Menorrhagia, Continuous flow. Particularly at climacteric. Haemorrhages from fibroids.

Dose—First to third potency.

(Materia Media—Boericke—P. 674.)

Following quotations are taken from Clinical Materia Medica by the Late E. A. Farrington M. D. P. P. 167.

'I find that Dr. Richard Hughes, in his Manual of Pharmacomynamics, speaks of three cases of post Climacteric haemorrhage, all of which were improved by vinca Minor. In one a permanent cure resulted; in the other two, there was a return of the haemorrhage, and in one of these the trouble was due to carcinoma'.

'It may be used for profuse menstruation or passive menorrhagia, when the blood flows in one continuous stream, associated of course with great debility' 34.

Summary and Conclusion

It is not unlikely that extensive and advanced researches of this interesting medicinal plant may bring out revolutionary changes in the chemo–theraphy and cure of different malignant and other kinds of diseases.

It is suggested that said drug may be tried Homoeopathically in different low potencies (1x, 2x, 3x to 12x and upto 30) as palliative on clinical and Experimental Research study work against diseases or syndromes like Leukaemia, Hodgkin's diseases, Menorrhagia, tumour, cancer breast and cervix etc. before proving.

Some of the the Medicinal plants which are reputedly used in folk medicine, Ayurvedic or Unani, seem to have great possibilities as Homoeopathic therapeutic agent specially in diseases where at present we do not have scientific record of a satisfactory response.

Before doing Homoeopathic proving of this drug we can do clinical use with this Homoeopathically prepared drug in the treatment of some one sided diseases for palliative and cnrative purpose and for clinical experimental research study in different low potencies and high potencies.

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Short Research Communication

CHEMICAL INVESTIGATION OF PYCNOCYCLA GLAUCA LINDL

Introduction

The plant *Pycnocycla glauca* Lindl. (Hindi–Tejraj) of family Umbelliferae has been reported from Satpura Hills and Chotanagpur area by Hooker[1] and Haines[2]. Recently the plant has been collected from one of the authors from Chauragarh Hill top near Pachmarhi of Satpura range. The root of the plant are used in Bihar as a remedy for dysentery.[3]

DESCRIPTION

The plant is a perennial herb, with woody root stock and is grey brown barked. Branched from the base, 24 cm to 150 cm high, striate, sub-glaucus. Radical leaves long petioled, pinnately dissected 1.4 cm to 7.5 cm, ultimate segments remote, glabrous or so; cauline, but smaller more shortly petioled. Umbels scabious or composite, heads 1.5 cm to 2.5 cm in diameter. Flowers white to purplish about 4.5 cm umbellate, bracts 5 to 6.8 cm; hairy, pedicels hairy; 7 to 8 males or nuters in two whorls envelope and enclose one fruit which attains .8 cm by .2 cm in size. Carpel with a T-shaped grove on the inner surface.

Specimen cited–NBG, F. B. No. 98733 collected by Peerzada and Joshi (illustratad). The specimen has been deposited in the Herbarium of the Institute.

In order to see if this plant can be of any use in medicine, chemical investigation of the whole plant was undertaken and the results are presented in this communication.

As a result βsitosterol, αspinstrerol and a triterpenic sapogenin, oleonolic acid have been isolated and identified from the plant.

Methods and Results

One kg. of the dried and powdered plant material was successively extracted with pet. –ether and methanol. The petrol-ether extract was concentrated and chromato-graphed over alumina using pet.-ether, benzene, chloroform and methanol as successive eluants.

The petrol ether eluate on fractional crystallization gave βsitosterol, $C_{29}H_{50}O$, the identity of which was confirmed through co-chromatography and super-imposable I. R. spectra with an authentic sample.

Benzene and benzene : chloroform fractions were mixed and saponified. The unsaponifiable matter on working up in the usual manner gave a compound mp. 167°, Liebraman-Burchardt test positive. The acetyl derivative when prepared in the usual manner melted at 187°C. The compound was identified to be αspinsterol (superimposable I.R., mmp and co-chromatography).

The defatted plant material was extracted with methanol in a soxhelet extractor. The methanol extract was hydrolysed with 4 per cent sulphuric acid. The genin was extracted with ether, which on purification through acid-alkali chromatography over acid washed

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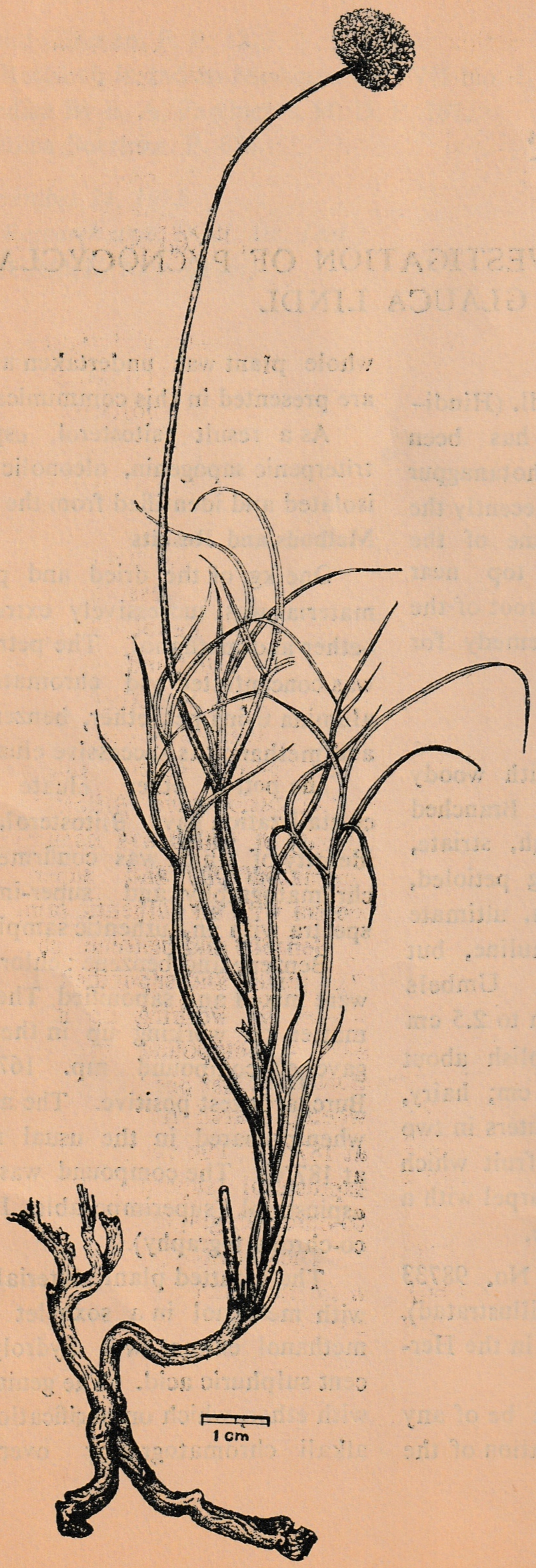

Pycnocycla Glauca

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alumina and finally on repeated crystallization from ethyl acetate; chloroform gave a pure sapogenin, mp. 306° (α) D = + 80° (chloroform) It shows all tests for triterpenoids. It showed infrared bands at 3332 (OH), 1688 (COOH), 1390 and 1462 (C–CH_3).

The sapogenin formed a mono acetate mp. 264° (α) D = + 76° (chloroform). The above data suggested the sapogenin to be oleonolic acid, the identity of which was further confirmed through mixed melting points and superimposable I.R. spectra with an authentic sample. The sugar was identified to be glucose and rhamnose by paper chromatography.

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Short Research Communication

EXPERIMENTAL STUDIES ON BHARANGI (*GARDENIA TURGIDA*), AN INDIGENOUS DRUG USED FOR THE TREATMENT OF BRONCHIAL ASTHMA

Introduction

The drug Bharangi of the Indian system of medicine is credited with several therapeutic properties, but its use is mainly indicated in Bronchial asthma. In the market several species of plants are sold under the vernacular name Bharangi and these include, *Gardenia turgida* (Fam. Rubiaceae), *Clerodendron serratum* (Fam. Verbenaceae), *Clerodendron siphonanthus* (Syn. Indicum), and *Picrasma quassioides* (Singh and Chuneker, 1972). of these the plant *Gardenia turgida* is most commonly sold in the local markets of the country as Bharangi and is extensively used as real Bharangi by the Ayurvedic practitioners in all preparations, where Bharangi is recommended in Ayurvedic pharmacopoeias (Bhatnagar *et al* , 1973).

Review of the modern literature shows that very little work has been done on the chemistry and pharmacology of *Gardenia turgida*. Forester *et al.* (19_5) reported the isolation of the active principle mannitol from *G. turgida*.

Ayurveda, however, recommends the use of the drug Bharangi for bronchial asthma especially (Charaka). In view thereof, a study has been undertaken to evaluate its effect on broncho–spasm in particular, alongwith some preliminary investigations on the effect of the drug on frog myocardium, ciliary movement and on the isolated smooth muscles of guineapigs.

Materials and methods

1. The drug was used as an alcoholic extract (80 gms. extract=1 kg. crude drug) in all the sets of experiments.

2. (a) Healthy guineapigs of both sexes weighing between 300 gms. to 400 gms. were used for evaluating the effect of the drug in preventing histamine induced broncho–spasm.

(b) Illeum strips isolated from healthy guineapigs was used for studying the effect of the drug on smooth muscles.

EXPERIMENT No. 1

Studies on Experimentally Induced Bronchial Spasm

This was done by the conventional method in the histamine aerosal chamber by spraying solution of histamine dihydrochloride at a pressure of 280 mm. of Hg.

TABLE 1

Observation of Histamine Reaction in Guineapigs.

No. of animals	Time taken for development of Bronchospasm (Asthma) in response to Histamine. Normal without drug treatment	$2\frac{1}{2}$ hrs. after treatment with drug.
1	68 seconds	81 seconds
2	19 seconds	68 seconds
3	90 seconds	98 seconds

From the above it has been seen that the drug does not provide any protection against histamine induced bronchospasm.

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EXPERIMENT No. 2

The Effect of Gardenia turgida Extractive on Frog Heart in Situ

This was estimated in the frog heart perfusion experiment in the usual manner. The drug was administered in graded doses as water suspension of alcoholic extract.

Results

The aquous solution of alcoholic extract of *G. turgida* when administered to the normodynamic heart showed that the smaller doses upto 5 mg. do not have any appreciablc effect. A dose of 10 mg. and subsequently higher dose of 15 mg. however induces a negative chronotropic and weakly positive inotropic effect on the heart.

A dose of 40 mg. effected complete block of cardiac movement for about 15 seconds which however, regained its normal activity with a negative chronotropic effect and a mild positive inotropic activity (Fig. 1).

The effect of the drug on hypodynamic heart showed that drug induced both negative chronotropic and negative inotropic effect as an immediate result of the administration of the drug. The force of contaction however returns to normal after sometime withou t correcting the rate of contraction (Fig. 2).

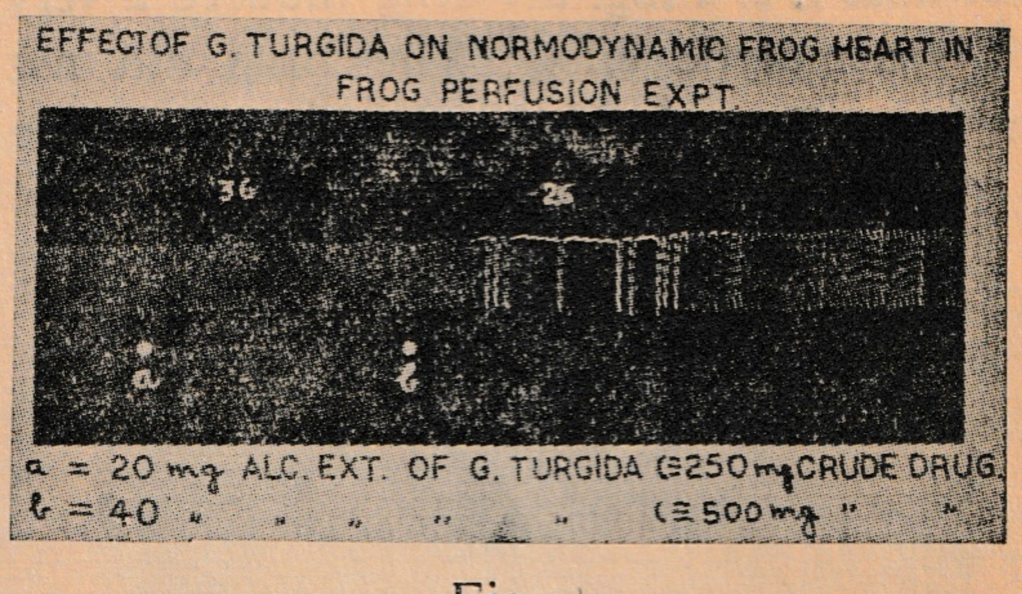

Fig.–1

A comparative evaluation of the effect of drug on normodynamic and hypodynamic heart showed that while the drug in the normodynamic heart initially caused a negative chromotropic and negative inotropic effect, the force of contraction (inotropic effect) however soon returns to normal but the effect no the hypodynamic heart was persistent negative chronotropic and negative inotropic action.

EXPERIMENT NO 3

To Study the Effect of Total *Alcoholic Extructs of Gardenia turgida on Isolated Guineapig Ileum : By the Conventional Method*

Results

The drug. *G. turgida* in doses of 1 and 2 mg. showed slight spasmodic effect but in the

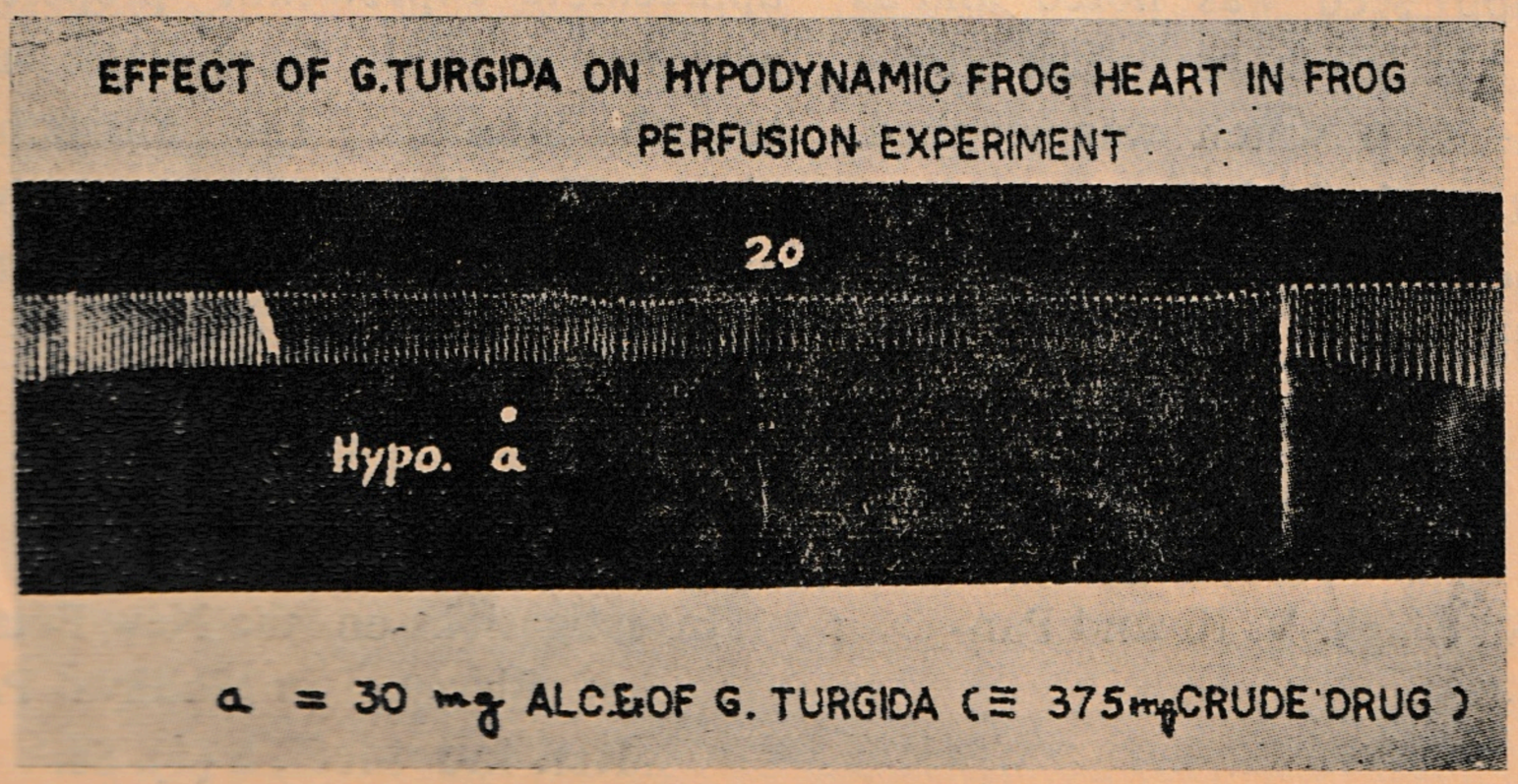

Fig.–2

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higher dose i. e. 4 mg. the drug induced a very weak spasmolytic effect. The drug did not seem to effect the acetylcholine response (Fig. 3).

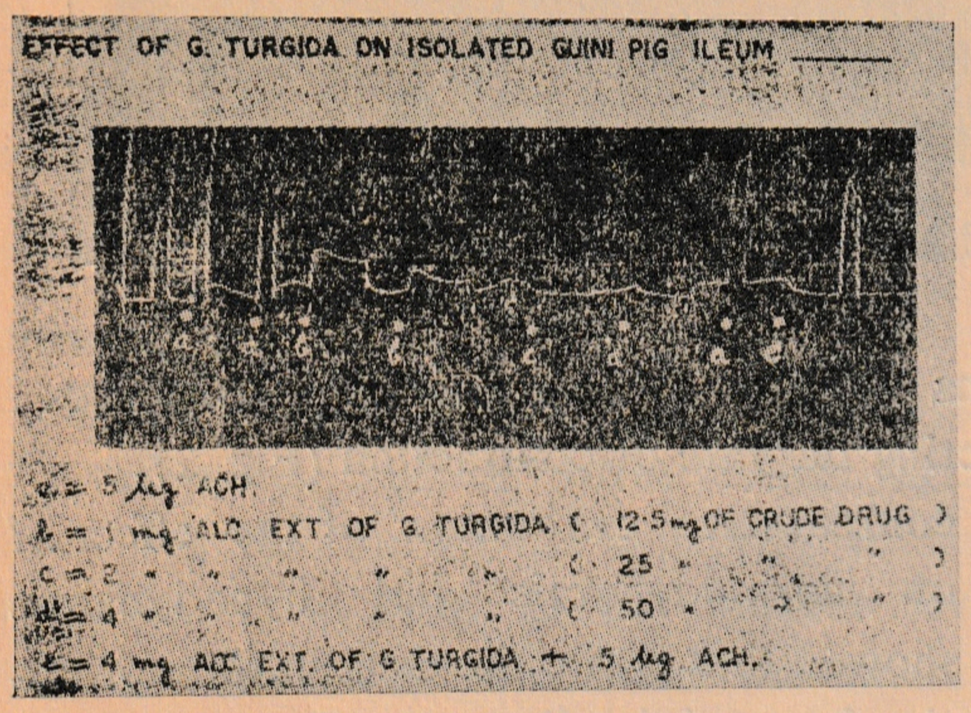

Fig.–3

EXPERIMENT NO. 4

Effect of Alcoholic Extracts of G. turgida on Frogs ciliary Movement : By a Modified Method in which the Laryngotrachial Chamber of the Frog was Exposed

Two points were marked on the exposed laryngotrachial chamber at a distance of about 2.5 cm. The area was washed with normal saline repeatedly to remove the mucus secretion completely. A grain of poppy seed was dropped at the upper mark near the glottis and stop watch was started. The gradual descent of the poppy seed was noted and as soon as it reached the lower mark just above the ligaments the stop watch was stopped. The area was next washed with aquous suspension of the alcoholic extract of the drug. The time taken for the poppy seed to travel the distance between the marks after drug treatment was noted.

TABLE 2

Effect tf G. turgida on Frog Ciliary Movement

No.	Time taken for poppy seed to travel the distance of two points (2.5 cm.)	
	Normal without drug treatment	After drug treatment
1	65 seconds	35 seconds
2	65 seconds	40 seconds
3	65 seconds	40 seconds
Mean	65 seconds	38.3 seconds

From the above it has been seen that the drug accelerates the ciliary movement of the frog. It means drug has some expectorant properties.

Conclusion

Experimental studies conducted with the drug *G. turgida*, sold in the market as Bharangi and recommended for treatment of Asthma in Indian system of medicine shows that it fails to prevent experimentally induced bronchospasm in guineapigs and does not possesses any antihistaminic effect. The drug however, seems to produce a negative chronotropic and a very weak inotropic effect. It has an appreciable expectorant property and also possesses a weak spasmolytic action on the smooth muscles. The antiasthmatic effect observed in clinical cases may be due to its expectorant and weak relaxant action on bronchial muscles.

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Jour. Res. Ind. Med. Yoga & Homoeo. 11: 4, 1976

Short Research Communication

IDENTIFICATION AND SEPARATION OF CHLOROPHYLLS FROM HOMOEOPATHIC MOTHER TINCTURE OF BELLADONNA

Introduction

The chlorophylls are a very important groups of metallic biomolecules responsible for transfer of radiant energy into chemical energy in autotrophic plants. As they have no therapeutic use in most of homoeopathic preparations there identification and separation from mother tinctures seems to be necessary. The present study has been undertaken to device a reliable method for identification and separation of chlorophylls in homoeopathic mother tinctures.

Materials and Methods

The tincture belladonna (I. P. & U. S. H. P., see ref. 3 & 4) has been used as a mterial for the present study. Chlorophylls were extracted by ethyl ether (5 : 20, three times). The solvent containing chlorophylls was concentrated to 2 ml. This concentrated extract was applied on cellulose TLC plate. The solvent system used was that of Bacon (1965) according to which petrolium ether (60–80), acetone, n-propanol (90 : 10 : 45), were used in a development distance of 15 cm. As soon as the solvent reached the upper edge the plates were removed and observed in natural and under U. V. light immediately.

0.02 ml of mother tincture devoid of chlorophylls, I. P. belladonna tincture and U. S. H. P. belladonna mother tincture, 0.01 ml (10 mg/50 ml) of reference standard hyoscyamine and hyoscine were separately spotted on silica gel plates to test the presence of alkaloids and chlorophylls. Benzene, ethylacetate, diethylamine in ratio of 7 : 2 : 1 were used as solvent in this case. After development the plate was dried and observed under U. V. light. Dragendorff's reagent was sprayed on the plate to visualise the spots of alkaloids.

The elute of chlorophyll a and b from cellulose TLC plate were tested for absorption spectra by the method of Dujardin *et al.* (1975).

Results

In cellulose plate five spots were visible. 4 th and 5 th spots of green colour were of chlorophyll a (Rf–75) and chlorophyll b (Rf–80). The elute of chlorphyll a and b from cellulose T. L. C. plate gave a absorption spectra between 400 mU and 500 mU and 600 and 700 mU respectively (Table 1).

In cochromatographic study on silicagel plates under U. V. light only the red spot (Rf–70–74) characteristic of chlorophylls was observed in the case of USHP belladonna mother tincture. Such a spot was not obeserved in the case of USHP tincture devoid of chlorophylls, IP belladonna tincture, reference standard hyoscyamine and hyoscine. The mother tincture USHP devoid of chlorophylls showed the two spots similar to the IP

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TABLE 1

Thin Layer Chromatography of Chlorophylls and Belladonna Alkaloids

Substances	Rf× 100	Colour A	Colour B
Chlorophyll derived from mother tincture USHP	70–74	Red	
Belladonna mother tincture USHP	45–49		Orange
	39–63		Orange
	70–74	Red	
Belladonna mother tincture devoid of chlorophylls	45–49		Orange
	59–63		Orange
Belladonna tincture IP	45–49		Orange
	59–63		Orange
Reference standard hyoscyamine	45–49		Orange
Reference standard hyoscine	59–63		Orange

Colour A :—Treatment under U. V. light.
Coiour B :—Detection with Dragendorff's reagent.

tincture out of which one was that of hyoscyamine (Rf 45–49) and the other of hyoscine (Rf 59–63)

Discussion

The methods of preparation of tinctures in homoeopathy and allopathy are almost same. But in allopathy the crude drugs are dried to a certain temperature so the chlorophylls are destroyed while in homoeopathy the fresh and green parts of the crude drugs are used as a result of which the chlorophylls are found to be present in mother tinctures. Therefore, there is a need of a procedure for the separation and identification of chlorophylls from homoeopathic mother tinctures.

As shown in the results all the active alkaloids of belladonna (hyoscyamine and hyoscine) were found to be present in the extract which were devoid of chlorophylls on silica gel plates. However, the original market extract revealed the presence of alkaloids as well as of chlorophylls on silica gel plates. The chlorophylls extracted from original market samples gave their characteristic spots on cellulose plate. Thus, by the above procedure chlorophylls can be separated and identified in homoeopathic mother tinctures.

It is to be mentioned here that the chlorophylls do have some therapeutic value because its molecule containes magnesium. But in most of the tinctures where the active ingredients are other than magnesium the chlorophylls should be considered as an adulterent meaning thereby that they should be separated from the standard mother tinctures to avoid the drug interaction. Thus the present study has an added pharmacognostical significance in deciding the drug standard of homoeopathic mother tinctures.

Summary and Conclusion

A method for separation and identification of chlorophylls from homoeopathic mother tinctures has been devised in the present study. It has been suggested that by the use of the above procedures the adulteration of chlorophylls in homoeopathic mother tinctures can be avoided.

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Short Research Communication

A NEW OBSERVATION ABOUT HAEMATINIC PROPERTY OF RAJATA BHASMA

Introduction

Rajata bhasma is one of the popular and useful therapeutic preparation in the indigeneous system of medicine. In Charaka Samhita poweder of the Rajata is claimed as rasayana[1] and anti asthmetic[2] agent. In Shushruta Samhita it is described as a universal antidote[3] and water disinfectant[4]. It is recommended in the treatment of conjunctivitis[5] and is a constituent of various anjana[6] preparations described in the text. In Vagbhata it is described as a brain tonic[7] and chief therapeutic preparation for the cases of food poisoning[8]. Astanga hridaya prescribes a paste[9] containing Rajata to remove the surgical scar.

Rasa literature and other authentic medical texts of Ayurveda mention a wide therapeutic use of Rajata bhasma which however is difficult to be explained according to modern concept. The ayurvedic practitioners of the present day however use Rajata bhasma only as a brain tonic and sexual stimulant

The present study has been under taken to assess its therapeutic claims in Ayurveda and accordingly its effect on central nervous system and on haemopoitic system have been studied in experimental animals.

Materials and Methods

Rajata bhasma—Rajata bhasma was prepared strictly under controlled condition according to the three methods described in Rastarangini (16/3, 16/1 and 16/6) and the product was chemically analysed to find out their chemical composition.

Their standerdised preparation was used in the experimental study. Forty albino rats weighing between 100 to 150 grms were taken. They were devided into four groups, each group consisting of ten rats. Individual weight of each rat in each group was recorded. The blood was taken from the tail vein from each of the animals and total R. B. C. Count, percentage of haemoglobin were estimated for each rat. The group No. I, II, III, served as experimental groups, who were given the test drugs by oral route in the form of linctus, prepared with honey and group No. IV was the Control group, who were given equivalent quantity of honey only. Animals of group No. I received rajata bhasma No. I (Prepared by rasa tarangini 19/3 method) in dose of 4 mg/100 gm, the animals of group No. II received rajata bhasma No. 2 (Prepared by rasa tarangini 16/1 method) in a dose of 4 mg/100 gm and the animals of group No. III received rajata bhasma No. 3 (Prepared by rasa tarangini 16/6 method) in a dose of 4 mg/ 100 gm. The drug was administered for fifteen days. On the seventeenth day blood was collected from each of the animals of all the four groups and the estimation of total R. B. C. and haemoglobin–percentage for each blood sample were repeated. The estimation of erythrocyte count and percentage of haemoglobin were done by the conventional methods.

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Obervations and Results

TABLE 1

Showing the effect of Rajata bhasma on total R. B. C. Count

S. No.	Group	No. of animals	Drug/Dose	R. B. C. Count NX 10,000 mill B. T.–A. T.	Remarks
1.	Control	10	Nil	+00.0–407.5	one expired
2.	Group I	10	R. B./4 mg/100 gm per day	426.6–556.6	
3.	Group II	10	do	387.3–592.1	one expired
4.	Group III	10	do	458 –628.8	

TABLE 2

Showing statistical analysis of total R. B.C Count

S. No.	Group	Mean (A. T.–B. T.) ± S. D.	Comparision of other groups with control	
1.	Control	3.25 ± 13.9		
2.	Group I	137.8 ± 101.9	t 6.13	P<0.001
3.	Group II	199 ± 112.8	t 5.65	P<0.001
4.	Group III	175.8 ± 111.7	t 5.21	P<0.001

TABLE 3

Showing the effect of Rajata bhasma on haemoglobin percentage

S. No.	Group	No. of Animals	Drug/Dose	Haemoglobin Percentage B. T.–A. T.	Remark
1.	Control	10	Nil	13.05–12.687	one expired
2.	Group I	10	R. B./4 mg/100 gm/ Perday	14.55–16.7	
3.	Group II	10	do	12.5 –15.44	one expired
4.	Group III	10	do	10.5 –16.7	

TABLE 4

Showing the statistical analysis of haemoglobin percentage

S. No.	Group	Mean (A. T.–B. T.)±S. D.	Comparision of other groups with control	
1.	Control	0. ±2.48		
2.	Group I	1.4 ±2.59	t 1.16	P>0.05
3.	Group II	2.125±3.78	t 1 328	P>0.05
4.	Group III	5.9 ±2.04	t 5.56	P<0.001

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Above said experimental studies on the effect of all the three samples of rajata bhasma on total R. B. C. count shows that all the three samples caused an increase in the total erthyrocyte count. However, studies of their effect on haemoglobin percentage showed that only sample No. III could increase the haemoglobin content of blood to an appreciable extent. This increase on statistical analysis shows to be highly significant (Table 1 & 2). This can very well account for the therapeutic use of rajata bhasma in conditions of anaemia. It should however be emphasised that of the three methods employed in the preparation of rajata bhasma the product obtained by ras tarangini 16/6 method was found to have a true haematinic effect.

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Short Research Communication

THE IDENTIFICATION OF PODOPHYLLUM ON PHARMACOGNOSTICAL CHEMICAL AND THIN LAYER CHROMATOGRAPHY

Introduction

The macroscopical and microscopical characters and results of preliminary chemical test, crude fibre determination and thin layer chromatographic examination of *Podophyllum peltatum* L. a commercial drug used as an adulterant with *Podophyllum hexandrum* R. have been described.

P. Peltatum L. (Berberidaceae) which is official both in Indian and United States Pharmacopoeias (see ref. 386) grows throughout United States and introduced into homoeopathy by Dr. Williamson in 1842.

The present study has been carried out with a view to clarify the root and rhizome of *P. peltatum* and *P. hexandrum* or Indian podophyllum. *P. hexandrum* is a herbaceous plant which grows in shady temperate forests of the Himalays from Sikim to Kashmir, The drug has long been used by the Indian vermitage and emetic.

DRUG CONSTITUENTS

The active principle of podophyllum is contained in resinous mixture known as Podophyllin. Podophyllotoxin, D–peltatin and other similar closely related compounds (Lignanes) are present in podophyllin. β Glucosides of them have also been isolated (Ramstad, 1959). *P. Peltatum* yields only 4 to 5 per cent resin, but *P. hexandrum* yields 8 to 13 per cent resin.

Podophyllotoxin obtained 2 to 4 per cent from *P. hexandrum* and 0.7 to 0.9 per cent from *P. Peltatum* (Chopra, 1958).

Experimental Materials

P. hexandrum was purchased from local market and *P. peltatum* was obtained from M/s. Wilhelm Kramer of West Germany. Both podophyllum mother tinctures were prepared in the laboratory according to U. S. H. P. (Ref. No. 6). The distinguising characters of the two roots and rhizomes studied are given below.

MACROSCOPICAL AND MICROSCOPICAL CHARACTERS

P. Peltaium occurs in sub-cylindrical reddish brown pieces about 5 to 20 cm long and 5 to 6 cm. thick. The outer surface is smooth (Autumn rhizome) or wrinkled (Summer rhizome). Slight odour and taste bitter and acrid. Transverse section of the rhizome shows a dark coloured epidermis, one or two layers of cork cells, a layer collenchymatus and parenchymatus cortex and pith. Many of thin cells contain reddish brown masses of resin cluster crystals of calcium oxalate and starch. The cluster crystals are 30 to 60 to100μ in diameter. (Wallis and Goldberg). *P. hexandrum*

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an earthy brown colour about 2 to 4 cm. long and 1 to 2 cm in diameter. The internodes are much shorter than in the *P. peltatum,* but the vascular bundles are more elongated radially. The calcium oxalate cluster are fewer and smaller 20 to 30 to 60μ (Wallis and Goldberg).

CRUDE FIBRE DETERMINATION

Determination of crude fibre (Goldberg and Wallis) value of *P. Peltatum* contains 7.5 per cent fibre, whereas *P. hexandrum* thus value is about 10.5 per cent.

CHEMICAL REACTION

Qualitative chemical tests are generally specific colour reactions are useful for identification. 5 per cent aqueous solution of eopper acetate which gives a bright green colour and no brown precipitate with an alcoholic solution of *P. peltatum* resin and a brown precipitate. but no green colour with an alcoholic solution of *P. Peltatu*m resin.

THIN LAYER CHROMATOGRAPHY

Preparation of the plates—A slurry of 25 g Silicagel G (E. Merck) in 50 ml of distilled water was applied to clear glass plates (20 × 20 × 0 3 cm.) at a predetermined thickness (0.25 mm) an adjustable spreader was used. The plates prepared in batches of five, were allowed to stand at room temperature for 5 minutes and dried in an oven at 100° for 30 minutes. 0.005 ml of alcoholic solution of *P. peltatum* and *P. hexandrum* applied on the plate and the solvent system (1st step chloroform, 10 per cent methanol. run 6 cm. from starting point and 2nd step chloroform, 35 per cent acetone run 12 cm from starting point).

After chromatogram the plates dried and sprayed with mixture of sulfuric acid and acetic anhydride 1 : 3, and warm for 15 min. at 100° (Stahl, 1965).

The T. L. C. characteristics of the *P. peltatum* showed four spots having Rf ranges 10-12, 18–20, 70–73 and 81–83. whereas *P. Hexandrum* showed eight spots having Rf ranges 10-12, 18-20, 25-28. 70-73, 81-83, 85-87, 88-90 and 95-97.

Summary and conclusion

A method for separation and identification of *P. Peltatum* has been devised in the present study. The T. L. C. method is particularly useful for distinguishing *P. hexandrum.* and its resin can be differentiated from *P. peltatum* resin on T. L. C. plates by multiple develop ment technique. It has been suggested that by the use of above procedure the adulteration of Podophyllum can be avoided.

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Short Research Communication

CHEMICAL EXAMINATION OF THE ROOTS OF PREMNA LATIFOLIA

Premna latifolia (Verbenaceae) is widely grown in the lower hills of coramondel coast and Bengal. The internal and external use of the leaves induces diuresis in cases of oedema. Milk of the bark is applied to boils and juice of the bark is given to cattle in colic[1]. The root is a constituent of the Ayurvedic preparation Dasamoola. No chemical work seems to have been done on this plant so far and so it was considered worthwhile to undertake a thorough investigation of the different parts of the plant used in indigenous system of medicine. The isolation and identification of compounds from the root are described in this paper, and the work on other parts of the plant will be published elsewhere.

Experimental

The dried and coarsely powdered root was completely extracted with hot ethanol. The alcohol extract on cooling deposited waxes which were removed. Further concentration and cooling deposited a little more wax and that was removed. This was repeated until no more wax was deposited. The solvent from the wax free extract was completely removed at low pressure and the sticky residue was refluxed with benzene repeatedly until the benzene extract did not answer the Libermann-Burchard test. The benzene extract was evaporated and the residue was saponified. The unsaponifiables were chromatographed over alumina. The compound obtained from the petroleum ether fraction was crystallised twice from methanol to get colourless crystals (mp. 85–86°C). It gave an acetate (mp. 73–74°C) with acetic anhydride and pyridine. Mixed melting point and co–T. L. C. with the authentic sample of n–triacontanol and its acetate confirmed the compound isolated was n–triacontanol. The compound obtained from the petroleum ether–benzene (1 : 1) fraction was crystallised from methanol. It melted at 135–137°C, gave an acetate (mp. 126–127°C) with acetic anhydride and pyridine. It has been identified as β–sitosterol by mixed melting point and co–T. L. C. with the authentic sample.

The benzene insoluble residue was extracted with dry ether until the ether extract was not positive to ferric chloride test. The ether extract was concentrated and extracted first with a saturated solution of sodium bicarbonate and then with 1% sodium hydroxide solution. They were acidified and taken in ether. The residues (very little) obtained from the ether solutions answered the general colour tests for flavonoids.[2] On paper chromatogram (Whatman No. 1, n–BaOH : AcOH : H_2O, 4 : 3 : 1) the residue from the bicarbonate fraction showed three bands while the residue from sodium hydroxide fraction showed one weak band, similar to one of the above three. Because of paucity of substance, further work could not be carried out.

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The ether insoluble residue was readily soluble in water and reduced the Fehling solution. It has been identified as d–glucose through its ozazone and cochromatogram with authentic sample.

Summary

The isolation and identification of n–triacontanol, β–sitosterol and d–glucose and the presence of three flavonoidal compounds in the roots of Premna latifolia are reported.

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Short Research Communication

IDENTIFICATION OF *EUPHORBIA TIRUCALLI* LINN

Euphorbia tirucalli (Family–Euphorbiaceae) commonly known as Septala, Chimia Sehud is well known for its medicinal use in the indigenous system of medicine (Kirtikar and Basu, 1933; Chopra et al, 1956). The green stems of this plant have marked resemblance with the stems of *Sarcostemma brevestigma* Wight Contrib (Family–Asclepiadaceae). As stems of both the species are green, glabrous, polished, terete with long internodes and hence their identification is difficult. In the present investigation a simple method of identification of *Euphorbia tirucalli* from *Sarcostemma brevestigma* on the basis of TLC pattern of their latex have been reported.

The latex from the two plant species was collected, air dried and 500 mg. of each was thoroughly extracted successively with petroleum ether (60--80°), benzene, chloroform and alcohol (90%) in a micro-soxhlet. The extracts were filtered, transfered, quantitatively to 25 ml. volumetric flask and volume was made to 25 ml. with respective solvents. 0.1 ml. of each extract was spotted on a dry silica gel-G (E. merck) plate and developed with benzene containing 0.1% of diethylamine. The visualization of spots was done using 1% sulphuric acid in alcohol followed by heating at 120° for 20 minntes. The results are tabulated below :—

Extracts	*Euphorbia tirucalli*		*Sarcostemma brevestigma*	
	No. of Spots	Rf	No. of Spots	Rf
Petroleum ether	Two	0.93 (M), 0.26 (M)	Six	0.93 (M), 0.81 (M), 0.65 (M), 0.53 (T), 0.23 (T), 0.13 (T).
Benzene	One	0.26 (T)	Seven	0.98 (T), 0.90 (M), 0.74 (M), 0.57 (M), 0.25 (T), 0.20 (T), 0.15 (T).
Chloroform	Three	0.98 (T), 0.70 (M), 0.25 (T).	Two	0.93 (T), 0.64 (M).
Alcohol	Nil	—	Nil	—

M—Major component.
T—Minor component.

From the table it is evident that TLC pattern of the different fractions of the latex of the two plant species is characteristic with marked differences, therefore, can be used as an easy means to their identification.

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Book Review

THE MADHAVANIDANA AND ITS CHIEF COMMENTARY
CHAPTERS 1–10
[Introduction, Translation and Notes]
GERRIT JAN MEULENBELD

This book was printed with financial support of the Netherlands Organization for the Advancement of Pure Research (Z. W. O.).

In the beginning, the book contains Table of contents, List of Abbreviations and Introduction (27 pages). Actual translation of Chapter 1 begins from the 29th page of the book and the last Chapter 10 ends at page 350. The Appendix one begins at page 357 and lastly Appendix five ends at page 629. From page 630, Bibliography starts and ends at page 645, further from page 646 Sanskrit Index starts and ends at page 668, followed by Index of Botanical names of plants which starts from page 669 and ends at page 688, still further page 689 and 690 deals with Index of Zoological names of Animals. Lastly, the book contains General Index which starts at page 691 and ends at page 709. In the end of the book, there are few lines about the author himself.

I read this translation with increasing fascination, absorption, and admiration. The timing of publication is fortunate, in view of the interest taken by Western medical men towards Ancient Indian Medical Science, in last few years.

After the age of the Samhitas, a large number of medical compendia and compilations were composed, out of them, most probably Mādhavanidāna is the first compilation of its type which appeared in the field of Sanskrit medical literature, still today it maintains its classical originality, as such has great importance as a book of diagnosis amongst Ayurvedic scholars. Although several attempts have been made to translate the Ayurvedic Samhitas into English but there was no any earlier attempt to translate this book of Sanskrit medical classics, which has its own place. It was the greatest need of the time to have an English Translation of Mādhavanidāna with its chief commentary, which has been partially fulfilled by Gerrit Jan Meulenbeld as he has been able to translate only first I0 Chapters of the book under review, The translation of this book has become more enlightening with the original notes, given by the translator.

The complete translation of Sanskrit medical texts with their commentaries in English, the international link language, is most justified and the need of the time, so that the ancient Indian Medical classics may be read, interpreted and utilised by most of the medical people, throughout the world, for the benefit of the suffering humanity. The attempt

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made by the translator is unique, in its type. It is complete so far the medical part is concerned, leaving apart the unrelivant parts of the commentary dealing with Sanskrit grammar. The Translator has tried to evolve and elaborate the translation technique to maintain the originality and consistency of the classic with its original commentary, and has also tried to keep the names of medicinal plants untranslated wherever their botanical identification is doubtful. He has been able to find out suitable English equivalents for the most of the technical Sanskrit terms, which generally lacks in earlier translations of Sanskrit medical classics, to make the translation easy for understanding the difference from the earlier translations the translator has given notes on the respective chapters which is advantageous that much part from hitherto untranslated texts can be presented.

The provision of appendices has increased the usefulness to those readers who have not made a specific study of Sanskrit medical classics. The concordance of the Nidāna and the lists of the quotations with their sources (App. 1), given by the author has completed the work; as the printed editions are not usually reliable and complete in this respect. He has also tried to correct the errors of the printed editions and filled up the gaps by tracing a number of hitherto unidentified passages and quotations, as far as possible. The translator has compiled the present available knowledge on the chronology of Sanskrit medical authors and their works (App. 2). Further, he has given some useful information on technical terms (App. 3), which has been collected from Sanskrit medical treatises and Nighantus for the benefit of historians and Sanskrits. Still further, he has also listed the plant names found (App. 4). An attempt has been made by the translator to identify the syndromes described or the clusters of symptoms enumerated in this volume (App. 5).

The dearth of published translations Sanskrit medical classics underscrores the great importance of the volume under review an informational venture aimed at making available to English speaking medical specialists, and the public at large the knowledge of ancient Indian Medical Classics. The book is not just impressive but impeccable. The earlier translations of Sanskrit medical classics, so far translated before this translation of Mādhavanidāna, have been itemized as 'recommended reading' or one part of the useful translations for the readers not knowing Sanskrit, but this particular translation of Mādhavanidāna has established its own authority by the evolvement of a new translation technique to maintain the originality and consistency of the classic with its original commentary. This translation must be read with some attention to understand it properly, because it is not easily digestible.

My first reaction on reading this volume was gratitude to the Jerrit Jan Meulenbeld for having conceived and successfully executed a plan to translate Sanskrit medical classics, like Mādhavanidāna alongwith its chief commentary. It is much to the credit of the author whose work is under review that he tried to avoid as far as possible over simplified, generalization and cliches and attempt instead to provide the reader with well-documented insights into the make up and contents of Mādhavanidāna and its chief commentary.

The Western reader also cannot fail to be impressed by the extent to which the knowledge of ancient Indian system of medicine, was developed, when they will read this English translation of Mādhavanidāna. The international opinion and interests of medical

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men now can be aroused towards Indian indigenous system of Medicine. Such efforts may mobilize public interest towards Ayurvedic system of medicine.

Gerrit Jan Meulenbeld's work is a prize gem; the medical specialist and non-medical specialist alike must be grateful to the translator for making this volume available The translator deserves the plaudits and the gratitude of many, whatever specialist interest involved, for launching this major enterprizes, which was long overdue.

In sum, the book under review is the work of serious seholarship and make important contributions, from different perspectives, to medical men knowledge of Sanskrit medical classics. The reviewer has found this translation instructive in the respect to induce interest in modern medical men to read, understand and utilize the ancient Indian medical knowledge, for the benefit and development of Modern medicine. The book is of post-graduate standard and, therefore, I recommend it for post-graduate scholars.

It will not be out of place to give a few lines about the author. Gerrit Jan Meulenbeld was born on May 28th, 1928, at Borne, the Netherlands. He attended the Christelijk Lyceum at Almelo where he passed the final examination Gymnasium B on June 17th, 1946. From 1946 he studied medicine at the University of Utrecht, in which subject he graduated in 1949 (candidaatsexamen), 1951 (doctoraal examen) and finally June 18th, 1954 (artsexamen). He specialized in psychiatry (October 16th, 1956–February 16th, 1959 and August 16th, 1960–October 16th 1961) with Dr, F. J. Tolsma (Deltaziekenhuis, Poortugaal) and in neurology (February 16 th, 1959–August 16th. 1960) with Prof. Dr. J. W. G. ter Braak (Westerzieken-huis and Ziekenhuis Dijkzigt, Rptterdam). He was registered as a specialist in mental and nervous diseases on October 16th, 1961. From that date on he is a member of the psychiatric staff of the Deltaziekeneuis, Poortugaal. During his medical training he studied Sanskrit with Prof. Dr. J. Gonda at the University of Utrecht.

This attempt of Jerrit Jan Meulenbeld is highly commondable.

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and
D. OJHA

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